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(54) Title: COMPOSITIONS AND METHODS FOR TREATING BONE DEFICIT CONDITIONS

(57) Abstract

Compounds containing two aromatic systems covalently linked through a linker containing one or more atoms, or "linker" defined as including a covalent bond *per se* so as to space the aromatic systems at a distance 1.5-15Å, are effective in treating conditions associated with bone deficits. The compounds can be administered to vertebrate subjects alone or in combination with additional agents that promote bone growth or that inhibit bone resorption. They can be screened for activity prior to administration by assessing their ability to effect the transcription of a reporter gene coupled to a promoter associated with a bone morphogenetic protein and/or their ability to stimulate calvarial growth in model animal systems.

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COMPOSITIONS AND METHODS FOR TREATING BONE DEFICIT CONDITIONS

Technical Field

The invention relates to compositions and methods for use in limiting undesired bone loss in a vertebrate at risk of such bone loss, in treating conditions that are characterized by undesired bone loss or by the need for bone growth, in treating fractures, and in treating cartilage disorders. More specifically, the invention concerns the use of specific classes of compounds identified or characterized by a high throughput screening assay.

Background Art

Bone is not a static tissue. It is subject to constant breakdown and resynthesis in a complex process mediated by osteoblasts, which produce new bone, and osteoclasts, which destroy bone. The activities of these cells are regulated by a large number of cytokines and growth factors, many of which have now been identified and cloned. Mundy has described the current knowledge related to these factors (Mundy, G.R. *Clin Orthop* 324:24-28, 1996; Mundy, G.R. *J Bone Miner Res* 8:S505-10, 1993).

Although there is a great deal of information available on the factors which influence the breakdown and resorption of bone, information on growth factors which stimulate the formation of new bone is more limited. Investigators have searched for sources of such activities, and have found that bone tissue itself is a storehouse for factors which have the capacity for stimulating bone cells. Thus, extracts of bovine bone tissue obtained from slaughterhouses contain not only structural proteins which are responsible for maintaining the structural integrity of bone, but also biologically active bone growth factors which can stimulate bone cells to proliferate. Among these latter factors are transforming growth factor ß, the heparin-binding growth factors (acidic and basic fibroblast growth factor), the insulin-like growth factors (insulin-like growth factor I and insulin-like growth factor II), and a recently described family of proteins called bone morphogenetic proteins (BMPs). All of these growth factors have effects on other types of cells, as well as on bone cells.

The BMPs are novel factors in the extended transforming growth factor ß superfamily. They were first identified by Wozney J. *et al. Science* (1988) 242:1528-34, using gene cloning techniques, following earlier descriptions characterizing the biological activity in extracts of demineralized bone (Urist M. *Science* (1965) 150:893-99). Recombinant BMP2 and BMP4 can induce new bone formation when they are injected locally into the subcutaneous tissues of rats (Wozney J. *Molec Reprod Dev* (1992) 32:160-67). These factors are expressed by normal osteoblasts as they differentiate, and have been shown to stimulate osteoblast differentiation and bone nodule formation *in vitro* as well as bone formation *in vivo* (Harris S. *et al. J. Bone Miner Res* (1994) 9:855-63). This latter property suggests potential usefulness as therapeutic agents in diseases which result in bone loss.

The cells which are responsible for forming bone are osteoblasts. As osteoblasts differentiate from precursors to mature bone-forming cells, they express and secrete a number of enzymes and structural proteins of the bone matrix, including Type-1 collagen, osteocalcin, osteopontin and alkaline phosphatase (Stein G. *et al. Curr Opin Cell Biol* (1990) 2:1018-27; Harris S. *et al.* (1994), *supra*). They also synthesize a number of growth regulatory peptides which are stored in the bone matrix, and are presumably responsible for normal bone formation. These growth regulatory peptides include the BMPs (Harris S. *et al.* (1994), *supra*). In studies of primary cultures of fetal rat calvarial osteoblasts, BMPs 1, 2, 3, 4, and 6 are expressed by cultured cells prior to the formation of mineralized bone nodules (Harris S. *et al.* (1994), *supra*). Like alkaline phosphatase, osteocalcin and osteopontin, the BMPs are expressed by cultured osteoblasts as they proliferate and differentiate.

Although the BMPs are potent stimulators of bone formation *in vitro* and *in vivo*, there are disadvantages to their use as therapeutic agents to enhance bone healing. Receptors for the bone morphogenetic proteins have been identified in many tissues, and the BMPs themselves are expressed in a large variety of tissues in specific temporal and spatial patterns. This suggests that BMPs may have effects on many tissues other than bone, potentially limiting their usefulness as therapeutic agents when administered systemically. Moreover, since they are peptides, they would have to be administered by injection. These disadvantages impose severe limitations to the development of BMPs as therapeutic agents.

There is a plethora of conditions which are characterized by the need to enhance bone formation. Perhaps the most obvious is the case of bone fractures, where it would be desirable to stimulate bone growth and to hasten and complete bone repair. Agents that enhance bone formation would also be useful in facial reconstruction procedures. Other bone deficit conditions include bone segmental defects, periodontal disease, metastatic bone disease, osteolytic bone disease and conditions where connective tissue repair would be beneficial, such as healing or regeneration of cartilage defects or injury. Also of great significance is the chronic condition of osteoporosis, including age-related osteoporosis and osteoporosis associated with post-menopausal hormone status. Other conditions characterized by the need for bone growth include primary and secondary hyperparathyroidism, disuse osteoporosis, diabetes-related osteoporosis, and glucocorticoid-related osteoporosis. In addition, or alternatively, the compounds of the present invention may modulate metabolism, proliferation and/or differentiation of normal or aberrant cells or tissues.

There are currently no satisfactory pharmaceutical approaches to managing any of these conditions. Bone fractures are still treated exclusively using casts, braces, anchoring devices and other strictly mechanical means. Further bone deterioration associated with post-menopausal osteoporosis has been decreased or prevented with estrogens or bisphosphonates.

US Patent 5, 280, 040 discloses a class of compounds which are 3, 4-diaryl chromans. These compounds can be considered derivatives of 2,3,4 triphenyl butanol, where the hydroxy at the 1-position forms an ether with the ortho position of the phenyl group substituted at the 4-position of the butanol. The parent 3,4-diaryl chromans do not contain nitrogen atoms in the aromatic moieties or their linkers. A preferred compound, centchroman, contains a nitrogen substituent only in one of the substituents on a phenyl moiety. These compounds are disclosed in the '040 patent as useful in the treatment of osteoporosis.

The present invention discloses compounds useful for limiting or treating bone deficit conditions, and for other uses that should be apparent to those skilled in the art from the teachings herein.

Disclosure of the Invention

The invention provides compounds that can be administered as ordinary pharmaceuticals and have the metabolic effect of enhancing bone growth. The compounds of the invention can be identified using an assay for their ability to activate control elements associated with these factors. Thus, the invention is directed to methods and compositions for stimulating the growth of skeletal (bone) tissue, which methods and compositions use, as active ingredients, compounds wherein two aromatic systems are coupled so as to be spaced apart from each other by about 1.5 to about 15 Angstroms. The thus-linked systems (including the linker coupling them) may include at least one nitrogen atom other than a ring substituent.

Therefore, the compounds useful in the invention can be described as having the formula Ar^1-linker-Ar^2, wherein each of Ar^1 and Ar^2 is independently an aromatic system and the linker portion of the formula spaces Ar^1 and Ar^2 apart by a distance of approximately 1.5-15 Angstroms. Ar^1, Ar^2 and the linker may optionally be substituted with non interfering substituents. In the useful compounds, there may be at least one nitrogen atom in either Ar^1, Ar^2 and/or the linker, independent of any substituents thereon. Preferably, the compounds of the invention also contain at least one additional heteroatom selected from the group consisting of N, S and O, independent of any substituent.

Other compounds of the invention include particular five membered rings having charge separation.

Thus, the invention is directed to methods to treat bone disorders using the compounds described and to pharmaceutical compositions for this use.

Brief Description of the Drawings

Figure 1 shows the dose response curve for the compound, designated 59-0008.

Figures 2 and 3 show illustrative compounds of the invention and the results obtained with them in an *in vitro* test.

Modes of Carrying Out the Invention

A rapid throughput screening test for compounds capable of stimulating expression of a reporter gene linked to a BMP promoter (a surrogate for the production of bone morphogenetic factors that are endogenously produced) is described in U.S. Application

Serial No. 08/458,434, filed 2 June 1995, the entire contents of which are incorporated herein by reference. This assay is also described as a portion of a study of immortalized murine osteoblasts (derived from a mouse expressing a transgene composed of a BMP2 promoter driving expression of T-antigen) in Ghosh-Choudhery, N. *et al. Endocrinology* (1996) 137:331-39. In this study, the immortalized cells were stably transfected with a plasmid containing a luciferase reporter gene driven by a mouse BMP2 promoter (-2736/114 bp), and responded in a dose-dependent manner to recombinant human BMP2.

Briefly, the assay utilizes cells transformed permanently or transiently with constructs in which the promoter of a bone morphogenetic protein, specifically BMP2 or BMP4, is coupled to a reporter gene, typically luciferase. These transformed cells are then evaluated for the production of the reporter gene product; compounds that activate the BMP promoter will drive production of the reporter protein, which can be readily assayed. Over 40,000 compounds have been subjected to this rapid screening technique, and only a very small percentage are able to elicit a level of production of luciferase 5-fold greater than that produced by vehicle. Compounds that activate the BMP promoter share certain structural characteristics not present in inactive compounds. The active compounds ("BMP promoter-active compounds" or "active compounds") are useful in promoting bone or cartilage growth, and thus in the treatment of vertebrates in need of bone or cartilage growth.

BMP promoter-active compounds can be examined in a variety of other assays that test specificity and toxicity. For instance, non-BMP promoters or response elements can be linked to a reporter gene and inserted into an appropriate host cell. Cytotoxicity can be determined by visual or microscopic examination of BMP promoter- and/or non-BMP promoter-reporter gene-containing cells, for instance. Alternatively, nucleic acid and/or protein synthesis by the cells can be monitored. For *in vivo* assays, tissues may be removed and examined visually or microscopically, and optionally examined in conjunction with dyes or stains that facilitate histologic examination. In assessing *in vivo* assay results, it may also be useful to examine biodistribution of the test compound, using conventional medicinal chemistry/animal model techniques.

As used herein, "limit" or "limiting" and "treat" or "treatment" are interchangeable terms. The terms include a postponement of development of bone deficit symptoms and/or a reduction in the severity of such symptoms that will or are expected to develop. The

terms further include ameliorating existing bone or cartilage deficit symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, preventing or reversing bone resorption and/or encouraging bone growth. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with a cartilage, bone or skeletal deficit, or with the potential to develop such deficit.

By "bone deficit" is meant an imbalance in the ratio of bone formation to bone resorption, such that, if unmodified, the subject will exhibit less bone than desirable, or the subject's bones will be less intact and coherent than desired. Bone deficit may also result from fracture, from surgical intervention or from dental or periodontal disease. By "cartilage defect" is meant damaged cartilage, less cartilage than desired, or cartilage that is less intact and coherent than desired.

Representative uses of the compounds of the present invention include: repair of bone defects and deficiencies, such as those occuring in closed, open and non-union fractures; prophylactic use in closed and open fracture reduction; promotion of bone healing in plastic surgery; stimulation of bone ingrowth into non-cemented prosthetic joints and dental implants; elevation of peak bone mass in pre-menopausal women; treatment of growth deficiencies; treatment of peridontal disease and defects, and other tooth repair processes; increase in bone formation during distraction osteogenesis; and treatment of other skeletal disorders, such as age-related osteoporosis, post-menopausal osteoporosis, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis. The compounds of the present invention can also be useful in repair of congenital, trauma-induced or surgical resection of bone (for instance, for cancer treatment), and in cosmetic surgery. Further, the compounds of the present invention can be used for limiting or treating cartilage defects or disorders, and may be useful in wound healing or tissue repair.

Bone or cartilage deficit or defect can be treated in vertebrate subjects by administering compounds of the invention which exhibit certain structural and functional characteristics. The compositions of the invention may be administered systemically or locally. For systemic use, the compounds herein are formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intraperitoneal, intranasal or transdermal) or enteral (e.g., oral or rectal) delivery according to conventional methods. Intravenous administration can be by a series of injections or by continuous infusion over an extended peri d. Administration by injection or ther routes of discretely spaced administration can

be performed at intervals ranging from weekly to once to three times daily. Alt rnatively, the compounds disclosed herein may be administered in a cyclical manner (administration of disclosed compound; followed by no administration; followed by administration of disclosed compound, and the like). Treatment will continue until the desired outcome is achieved. In general, pharmaceutical formulations will include a compound of the present invention in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water, borate-buffered saline containing trace metals or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, lubricants, fillers, stabilizers, etc. Methods of formulation are well known in the art and are disclosed, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton PA, 1990, which is incorporated herein by reference. Pharmaceutical compositions for use within the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art. Local administration may be by injection at the site of injury or defect, or by insertion or attachment of a solid carrier at the site, or by direct, topical application of a viscous liquid, or the like. For local administration, the delivery vehicle preferably provides a matrix for the growing bone or cartilage, and more preferably is a vehicle that can be absorbed by the subject without adverse effects.

Delivery of compounds herein to wound sites may be enhanced by the use of controlled-release compositions, such as those described in pending U.S. Patent Application No. 07/871,246 (corresponding to WIPO publication WO 93/20859, which is incorporated herein by reference in its entirety). Films of this type are particularly useful as coatings for prosthetic devices and surgical implants. The films may, for example, be wrapped around the outer surfaces of surgical screws, rods, pins, plates and the like. Implantable devices of this type are routinely used in orthopedic surgery. The films can also be used to coat bone filling materials, such as hydroxyapatite blocks, demineralized bone matrix plugs, collagen matrices and the like. In general, a film or device as described herein is applied to the bone at the fracture site. Application is generally by implantation into the bone or attachment to the surface using standard surgical procedures.

In addition to the copolymers and carriers noted above, the biodegradable films and matrices may include other active or inert components. Of particular interest are those

agents that promote tissue growth or infiltration, such as growth factors. Exemplary growth factors for this purpose include epidermal growth fact r (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factors (TGFs), parathyroid hormone (PTH), leukemia inhibitory factor (LIF), and insulin-like growth factors (IGFs) and the like. Agents that promote bone growth, such as bone morphogenetic proteins (U.S. Patent No. 4,761,471; PCT Publication WO 90/11366), osteogenin (Sampath *et al. Proc. Natl. Acad. Sci. USA* (1987) 84:7109-13) and NaF (Tencer *et al. J. Biomed. Mat. Res.* (1989) 23: 571-89) are also preferred. Biodegradable films or matrices include calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyanhydrides, bone or dermal collagen, pure proteins, extracellular matrix components and the like and combinations thereof. Such biodegradable materials may be used in combination with non-biodegradable materials, to provide desired mechanical, cosmetic or tissue or matrix interface properties.

Alternative methods for delivery of compounds of the present invention include use of ALZET osmotic minipumps (Alza Corp., Palo Alto, CA); sustained release matrix materials such as those disclosed in Wang *et al.* (PCT Publication WO 90/11366); electrically charged dextran beads, as disclosed in Bao *et al.* (PCT Publication WO 92/03125); collagen-based delivery systems, for example, as disclosed in Ksander *et al. Ann. Surg.* (1990) 211(3):288-94; methylcellulose gel systems, as disclosed in Beck *et al. J. Bone Min. Res.* (1991) 6(11):1257-65; and alginate-based systems, as disclosed in Edelman *et al. Biomaterials* (1991) 12:619-26 and the like. Other methods well known in the art for sustained local delivery in bone include porous coated metal protheses that can be impregnated and solid plastic rods with therapeutic compositions incorporated within them.

The compounds of the present invention may also be used in conjunction with agents that inhibit bone resorption. Antiresorptive agents, such as estrogen, bisphosphonates and calcitonin, are preferred for this purpose. More specifically, the compounds disclosed herein may be administered for a period of time (for instance, months to years) sufficient to obtain correction of a bone deficit condition. Once the bone deficit condition has been corrected, the vertebrate can be administered an anti-resorptive compound to maintain the corrected bone condition. Alternatively, the compounds disclosed herein may be adminstered with an anti-resorptive compound in a cyclical manner

(administration of disclosed compound, followed by anti-res rptive, followed by disclosed compound, and the like).

In additional formulations, conventional preparations such as those described below may be used.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin, lysolecithin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure.

If desired, the osteogenic agents can be incorporated into liposomes by any of the reported methods of preparing liposomes for use in treating various pathogenic conditions. The present compositions may utilize the compounds noted above incorporated in liposomes in order to direct these compounds to macrophages, monocytes, other cells and tissues and organs which take up the liposomal composition. The liposome-incorporated compounds of the invention can be utilized by parenteral administration, to allow for the efficacious use of lower doses of the compounds. Ligands may also be incorporated to further focus the specificity of the liposomes.

Suitable conventional methods of liposome preparation include, but are not limited to, those disclosed by Bangham, A.D. *et al. J Mol Biol* (1965) 23:238-252, Olson, F. *et al. Biochim Biophys Acta* (1979) 557:9-23, Szoka, F. *et al. Proc Natl Acad Sci USA* (1978) 75:4194-4198, Mayhew, E. *et al.* _____ (1984) 775:169175, Kim, S. *et al. Biochim Biophys Acta* (1983) 728:339:348, and Mayer, *et al. Biochim Biophys Acta* (1986) 858:161-168.

The liposomes may be made from the present compounds in combination with any of the conventional synthetic or natural phospholipid liposome materials including phospholipids from natural sources such as egg, plant or animal sources such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, or phosphatidylinositol. Synthetic phospholipids that may also be used, include, but are not limited to: dimyristoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidycholine, and the corresponding synthetic phosphatidylethanolamines and phosphatidylglycerols. Cholesterol or other sterols, cholesterol hemisuccinate, glycolipids, cerebrosides, fatty acids, gangliosides, sphingolipids, 1,2-bis(oleoyloxy)-3-(trimethyl ammonio) propane (DOTAP), N-[1-(2,3-dioleoyl) propyl-N,N,N-trimethylammonium chloride (DOTMA), and other cationic lipids may be incorporated into the liposomes, as is known to those skilled in the art. The relative amounts of phospholipid and additives used in the liposomes may be varied if desired. The preferred ranges are from about 60 to 90 mole percent of the phospholipid; cholesterol, cholesterol hemisuccinate, fatty acids or cationic lipids may be used in amounts ranging from 0 to 50 mole percent. The amounts of the present compounds incorporated into the lipid layer of liposomes can be varied with the concentration of the lipids ranging from about 0.01 to about 50 mole percent.

Using conventional methods, approximately 20 to 30% of the compound present in solution can be entrapped in liposomes; thus, approximately 70 to 80% of the active compound is wasted. In contrast, where the compound is incorporated into liposomes, virtually all of the compound is incorporated into the liposome, and essentially none of the active compound is wasted.

The liposomes with the above formulations may be made still more specific for their intended targets with the incorporation of monoclonal antibodies or other ligands specific for a target. For example, monoclonal antibodies to the BMP receptor may be incorporated into the liposome by linkage to phosphatidylethanolamine (PE) incorporated into the liposome by the method of Leserman, L. *et al. Nature* (1980) 288:602-604.

Veterinary uses of the disclosed compounds are also contemplated. Such uses would include limitation or treatment of bone or cartilage deficits or defects in domestic animals, livestock and thoroughbred horses. The compounds described herein can also

modify a target tissue or organ environment, so as to attract bone-forming cells to an environment in need of such cells.

The compounds of the present invention may also be used to stimulate growth of bone-forming cells or their precursors, or to induce differentiation of bone-forming cell precursors, either *in vitro* or *ex vivo*. As used herein, the term "precursor cell" refers to a cell that is committed to a differentiation pathway, but that generally does not express markers or function as a mature, fully differentiated cell. As used herein, the term "mesenchymal cells" or "mesenchymal stem cells" refers to pluripotent progenitor cells that are capable of dividing many times, and whose progeny will give rise to skeletal tissues, including cartilage, bone, tendon, ligament, marrow stroma and connective tissue (see A. Caplan *J. Orthop. Res.* (1991) 9:641-50). As used herein, the term "osteogenic cells" includes osteoblasts and osteoblast precursor cells. More particularly, the disclosed compounds are useful for stimulating a cell population containing marrow mesenchymal cells, thereby increasing the number of osteogenic cells in that cell population. In a preferred method, hematopoietic cells are removed from the cell population, either before or after stimulation with the disclosed compounds. Through practice of such methods, osteogenic cells may be expanded. The expanded osteogenic cells can be infused (or reinfused) into a vertebrate subject in need thereof. For instance, a subject's own mesenchymal stem cells can be exposed to compounds of the present invention *ex vivo*, and the resultant osteogenic cells could be infused or directed to a desired site within the subject, where further proliferation and/or differentiation of the osteogenic cells can occur without immunorejection. Alternatively, the cell population exposed to the disclosed compounds may be immortalized human fetal osteoblastic or osteogenic cells. If such cells are infused or implanted in a vertebrate subject, it may be advantageous to "immunoprotect" these non-self cells, or to immunosuppress (preferably locally) the recipient to enhance transplantation and bone or cartilage repair.

Within the present invention, an "effective amount" of a composition is that amount which produces a statistically significant effect. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an active compound herein required to provide a clinically significant increase in healing rates in fracture repair; reversal of bone loss in osteoporosis; reversal of cartilage defects or disorders; prevention or delay of onset of osteoporosis; stimulation and/or augmentation of bone formation in

fracture non-unions and distraction osteogenesis; increase and/or acceleration of bone growth into prosthetic devices; and repair of dental defects. Such effective amounts will be determined using routine optimization techniques and are dependent on the particular condition to be treated, the condition of the patient, the route of administration, the formulation, and the judgment of the practitioner and other factors evident to those skilled in the art. The dosage required for the compounds of the invention (for example, in osteoporosis where an increase in bone formation is desired) is manifested as a statistically significant difference in bone mass between treatment and control groups. This difference in bone mass may be seen, for example, as a 5-20% or more increase in bone mass in the treatment group. Other measurements of clinically significant increases in healing may include, for example, tests for breaking strength and tension, breaking strength and torsion, 4-point bending, increased connectivity in bone biopsies and other biomechanical tests well known to those skilled in the art. General guidance for treatment regimens is obtained from experiments carried out in animal models of the disease of interest.

The dosage of the compounds of the invention will vary according to the extent and severity of the need for treatment, the activity of the administered compound, the general health of the subject, and other considerations well known to the skilled artisan. Generally, they can be administered to a typical human on a daily basis on an oral dose of about 0.1 mg/kg-1000 mg/kg, and more preferably from about 1 mg/kg to about 200 mg/kg. The parenteral dose will appropriately be 20-100% of the oral dose.

Screening Assays

The osteogenic activity of the compounds used in the methods of the invention can be verified using *in vitro* screening techniques, such as the assessment of transcription of a reporter gene coupled to a bone morphogenetic protein-associated promoter, as described above, or in alternative assays such as the following:

Technique for Neonatal Mouse Calvaria Assay (*In vitro*)

This assay is similar to that described by Gowen M. & Mundy G. *J Immunol* (1986) 136:2478-82. Briefly, four days after birth, the front and parietal bon s of ICR Swiss white mouse pups are removed by microdissection and split along the sagittal suture. The bones are incubated in BGJb medium (Irvine Scientific, Santa Ana, CA) plus 0.02% (or lower

concentration) β-methylcyclodextrin, wherein the medium also contains test or control substances, at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 96 hours.

Following this, the bones are removed from the incubation media and fixed in 10% buffered formalin for 24-48 hours, decalcified in 14% EDTA for 1 week, processed through graded alcohols; and embedded in paraffin wax. Three μm sections of the calvaria are prepared. Representative sections are selected for histomorphometric assessment of bone formation and bone resorption. Bone changes are measured on sections cut 200 μm apart. Osteoblasts and osteoclasts are identified by their distinctive morphology.

Other auxillary assays can be used as controls to determine non-BMP promoter-mediated effects of test compounds. For example, mitogenic activity can be measured using screening assays featuring a serum-response element (SRE) as a promoter and a luciferase reporter gene. More specifically, these screening assays can detect signalling through SRE-mediated pathways, such as the protein kinase C pathway. For instance, an osteoblast activator SRE-luciferase screen and an insulin mimetic SRE-luciferase screen are useful for this purpose. Similarly, test compound stimulation of cAMP response element (CRE)-mediated pathways can also be assayed. For instance, cells transfected with receptors for PTH and calcitonin (two bone-active agents) can be used in CRE-luciferase screens to detect elevated cAMP levels. Thus, the BMP promoter specificity of a test compound can be examined through use of these types of auxillary assays.

In vivo Assay of Effects of Compounds on Murine Calvarial Bone Growth

Male ICR Swiss white mice, aged 4-6 weeks and weighing 13-26 gm, are employed, using 4-5 mice per group. The calvarial bone growth assay is performed as described in PCT application WO 95/24211, incorporated by reference. Briefly, the test compound or appropriate control vehicle is injected into the subcutaneous tissue over the right calvaria of normal mice. Typically, the control vehicle is the vehicle in which the compound was solubilized, and is PBS containing 5% DMSO or is PBS containing Tween (2 μl/10 ml). The animals are sacrificed on day 14 and bone growth measured by histomorphometry. Bone samples for quantitation are cleaned from adjacent tissues and fixed in 10% buffered formalin for 24-48 hours, decalcified in 14% EDTA for 1-3 weeks, processed through graded alcohols; and embedded in paraffin wax. Three to five μm

sections of the calvaria are prepared, and representative sections are selected for histomorphometric assessment of the effects on bone formation and bone resorption. Sections are measured by using a camera lucida attachment to trace directly the microscopic image onto a digitizing plate. Bone changes are measured on sections cut 200 μm apart, over 4 adjacent 1x1 mm fields on both the injected and noninjected sides of the calvaria. New bone is identified by its characteristic woven structure, and osteoclasts and osteoblasts are identified by their distinctive morphology. Histomorphometry software (OsteoMeasure, Osteometrix, Inc., Atlanta) is used to process digitizer input to determine cell counts and measure areas or perimeters.

Additional *In Vivo* Assays

Lead compounds can be further tested in intact animals using an *in vivo*, dosing assay. Prototypical dosing may be accomplished by subcutaneous, intraperitoneal or oral administration, and may be performed by injection, sustained release or other delivery techniques. The time period for administration of test compound may vary (for instance, 28 days as well as 35 days may be appropriate). An exemplary, *in vivo* subcutaneous dosing assay may be conducted as follows:

In a typical study, 70 three-month-old female Sprague-Dawley rats are weight-matched and divided into seven groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; a control group administered vehicle only; a PBS-treated control group; and a positive control group administered a compound (non-protein or protein) known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups.

Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. All animals are injected with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day). Weekly body weights are determined. At the end of the 35-day cycle, the animals are weighed and bled by orbital or cardiac puncture. Serum calcium, phosphate, osteocalcin, and CBCs are determined. Both leg bones (femur and tibia) and lumbar vertebrae are removed, cleaned of adhering soft tissue, and stored in 70% ethanol for evaluation, as performed by peripheral quantitative computed tomography (pQCT; Ferretti,

J. *Bone* (1995) 17:353S-64S), dual energy X-ray absorptiometry (DEXA; Laval-Jeantet A. *et al. Calcif Tissue Intl* (1995) 56:14-18; J. Casez *et al. Bone and Mineral* (1994) 26:61-68) and/or histomorphometry. The effect of test compounds on bone remodeling can thus be evaluated.

Lead compounds can also be tested in acute ovariectomized animals (prevention model) using an *in vivo* dosing assay. Such assays may also include an estrogen-treated group as a control. An exemplary subcutaneous dosing assay is performed as follows:

In a typical study, 80 three-month-old female Sprague-Dawley rats are weight-matched and divided into eight groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; three control groups (sham ovariectomized (sham OVX) + vehicle only; ovariectomized (OVX) + vehicle only; PBS-treated OVX); and a control OVX group that is administered a compound known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups of OVX animals.

Since ovariectomy (OVX) induces hyperphagia, all OVX animals are pair-fed with sham OVX animals throughout the 35 day study. Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. Alternatively, test compound can be formulated in implantable pellets that are implanted for 35 days, or may be administered orally, such as by gastric gavage. All animals, including sham OVX/vehicle and OVX/vehicle groups, are injected intraperitoneally with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day, to ensure proper labeling of newly formed bone). Weekly body weights are determined. At the end of the 35-day cycle, the animals' blood and tissues are processed as described above.

Lead compounds may also be tested in chronic OVX animals (treatment model). An exemplary protocol for treatment of established bone loss in ovariectomized animals that can be used to assess efficacy of anabolic agents may be performed as follows. Briefly, 80 to 100 six month old female, Sprague-Dawley rats are subjected to sham surgery (sham OVX) or ovariectomy (OVX) at time 0, and 10 rats are sacrificed to serve as baseline controls. Body weights are recorded weekly during the experiment. After approximately 6 weeks of bone depletion (42 days), 10 sham OVX and 10 OVX rats are randomly selected for sacrifice as depletion period controls. Of the remaining animals, 10 sham OVX and 10

OVX rats are used as placebo-treated controls. The remaining OVX animals are treated with 3 to 5 doses of test drug for a period of 5 weeks (35 days). As a postitive control, a group of OVX rats can be treated with an agent such as PTH, a known anabolic agent in this model (Kimmel *et al. Endocrinology* (1993) 132:1577-84). To determine effects on bone formation, the following procedure can be followed. The femurs, tibiae and lumbar vertebrae 1 to 4 are excised and collected. The proximal left and right tibiae are used for pQCT measurements, cancellous bone mineral density (BMD) (gravimetric determination), and histology, while the midshaft of each tibiae is subjected to cortical BMD or histology. The femurs are prepared for pQCT scanning of the midshaft prior to biomechanical testing. With respect to lumbar vertebrae (LV), LV2 are processed for BMD (pQCT may also be performed); LV3 are prepared for undecalcified bone histology; and LV4 are processed for mechanical testing.

Nature of the Compounds Useful in the Invention

All of the compounds of the invention contain two aromatic systems, Ar^1 and Ar^2, spaced apart by a linker at a distance of 1.5-15Å, and may contain at least one nitrogen atom. Both the systems represented by Ar^1 and Ar^2 may contain non-interfering substituents. The non-interfering substituents on the aromatic system represented by Ar^1 and the non-interfering substituents on the aromatic system represented by Ar^2 are represented in the formulae herein by R^a and R^b, respectively; however, it is recognized that the designation of one Ar as Ar^1 and the other as Ar^2 is arbitrary. For ease of reference, each is designated separately; it will, however, be evident that the linkers described below, unless palindromic, could thus exist in the compounds in "reverse" order of atoms. Generally, the non-interfering substituents can be of wide variety. Among substituents that do not interfere with the beneficial effect of the compounds of the invention on bone in treated subjects are included alkyl (1-6C, preferably lower alkyl 1-4C), including straight or branched-chain forms thereof, alkenyl (1-6C, preferably 1-4C), alkynyl (1-6C, preferably 1-4C), all of which can be straight or branched chains and may contain further substituents; halogens, including F, Cl, Br and I; siloxy, OR, SR, NR_2, OOCR, COOR, NCOR, NCOOR, and benzoyl, CF_3, OCF_3, SCF_3, $N(CF_3)_2$, CN, SO, SO_2R and SO_3R wherein R is alkyl (1-6C) or is H. Where two R^a or two R^b substituents are in adjacent positions in the

aromatic system, they may form a ring. Further, rings may be included in substituents which contain sufficient carbon atoms and heteroatoms to provide this possibility.

Preferred non-interfering substituents include hydrocarbyl groups f 1-6C, including saturated and unsaturated, linear or branched hydrocarbyl as well as hydrocarbyl groups containing ring systems; halo groups, alkoxy, hydroxy, amino, monoalkyl- and dialkylamino where the alkyl groups are 1-6C, CN, CF_3, and COOR.

Although the number of R^a and R^b substituents may typically be 0-4 or 0-5 depending on the available positions in the aromatic system, preferred embodiments include those wherein the number of R^a is 0, 1 or 2 and of R^b is 0, 1 or 2.

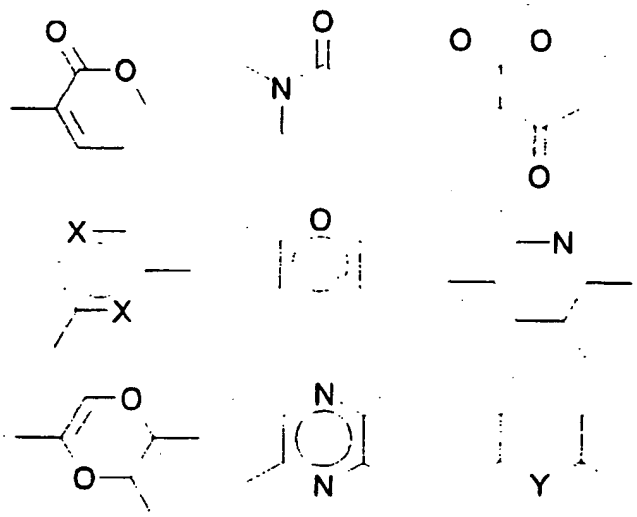
The linker group, L, may be a covalent bond or any group having a valence of at least two and covering a linear distance of from about 1.5 to about 15 Angstroms, including those that contain cyclic moieties, that meet this spatial requirement. Useful linkers are divided, by definition herein, into three general categories: (1) flexible non-conjugating linkers, (2) flexible conjugating linkers, and (3) constrained linkers. The preferred choice of linker will depend on the choices for Ar^1 and Ar^2. Not all of the linkers defined below are suitable for all Ar^1 and Ar^2 combinations.

As defined herein, *flexible non-conjugating* linkers are those that link only one position of Ar^1 to one position of Ar^2, and provide only a single covalent bond or a single chain between Ar^1 and Ar^2. The chain may contain branches, but may not contain π-bonds (except in the branches) or cyclic portions in the chain. The linker atoms in the chain itself rotate freely around single covalent bonds, and thus the linker has more than two degrees of freedom. Particularly useful flexible non-conjugating linkers, besides a covalent bond, are those of the formulae: -NR-, $-CR_2-$, -S-, or -O-, wherein R is H or alkyl (1-6C), more preferably H or lower alkyl (1-4C) and more preferably H. Also preferred are those of the formulae: -NRCO-, -CONR-, $-CR_2S-$, $-SCR_2-$, $-OCR_2-$, $-CR_2O-$, -NRNR-, $-CR_2CR_2-$, $-NRSO_2-$, $-SO_2NR-$, $-CR_2CO-$, $-COCR_2-$, and -NR-NR-CO-CR_2- and its complement $-CR_2$-CO-NR-NR-, including the isosteres thereof. Also preferred are those of the formulae: $-NR(CR_2)_2NR-$, $-O(CR_2)_2O-$, and $-S(CR_2)_2S-$, including the isosteres thereof. The optimum choice of linker within this group is dependent on the nature of Ar^1 and Ar^2.

Flexible conjugating linkers are those that link only one position of Ar^1 to one position of Ar^2, but incorporate at least one double or triple bond and/or one or more cyclic systems and thus have only two degrees of freedom. A flexible conjugating linker may

form a completely conjugated π-bond linking system between Ar^1 and Ar^2, thus providing for co-planarity of Ar^1 and Ar^2. Examples of useful flexible conjugating linkers include: -RC=CR-; -N=N-; -C≡C-; -RC=N-; -N=CR-; -NR-N=CR-; -NR-NR-CO-CR=CR-; and the like, where R is H or alkyl (1-6C); preferably H or lower alkyl (1-4C); and more preferably H.

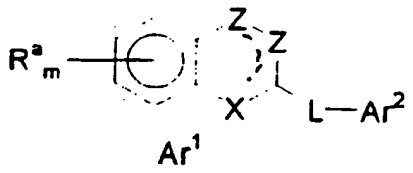
Constrained linkers are those that have more than one point of attachment to either or both Ar^1 and Ar^2 and, thus, generally allow for only one degree of freedom. Constrained linkers most frequently form fused 5- or 6-membered cyclic moieties with Ar^1 and/or Ar^2 where either Ar^1 or Ar^2 has at least one substituent appropriately positioned to form a second covalent bond with the linker, e.g., where Ar^2 is a phenyl group with a reactive, ortho-positioned substituent, or is derivatized to the linker directly at the ortho position. (Although the aromatic moieties should properly be referred to as phenylene or naphthylene in such cases, generally the term "phenyl" or "naphthyl" is used herein to include both monovalent and bivalent forms of these moieties.) Examples of particularly useful constrained linkers include



and the like, where X is O, N, S or CR, and Y is CR_2 or C=O.

Many of the compounds useful in the invention are commercially available and can be synthesized by art-known methods. Those compounds useful in the invention which are new compounds, can similarly be obtained by methods generally known in the art.

In one set of compounds of the inventions, Ar^1 is a substituted or unsubstituted aromatic system containing a six-membered heterocycle and the compounds useful in the invention have the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

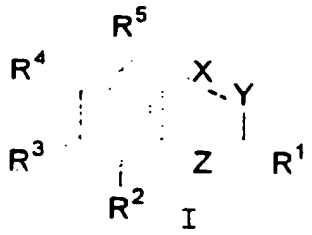
each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

A particularly preferred set of embodiments is of the formula:



in which:

R^1 is taken from the group: N=NAr, NR^6COAr, $CONR^6Ar$, CH_2OAr, CH_2NR^6Ar, where Ar is a six-membered (un)substituted aromatic ring. Allowable substituents on this aromatic ring include:

halogen, straight or branched chain lower alkyl, alkenyl, or alkynyl, optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, siloxy, acyloxy, straight or branched chain lower alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy, in which

R^6 is taken from the group: hydrogen, or straight or branched chain lower alkyl;

R^2 and R^5 are individually taken from the group: H, hydroxy, siloxy, acyloxy, halo, cyano, straight or branched chain lower alkyl, or straight or branched chain lower alkoxyl;

R^3 and R^4 are individually taken from the group: H, halogen, straight or branched chain lower alkyl, alkenyl, or alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, siloxy, acyloxy, straight or branched chain lower alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, and carboxy;

X and Y are either: NR^8 and N, respectively, in which case X and Y are singly bonded, or CR^9 and CR^{10}, respectively, in which case X and Y are doubly bonded, wherein

R^8 is either H or lower alkyl;

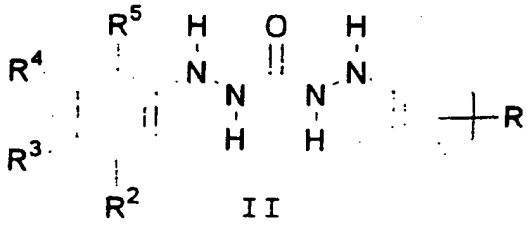
R^9 and R^{10} are individually taken from the group: H, halo, and lower alkyl;

Z is taken from the group: O, S, SO, and SO_2;

or salts thereof.

Compounds of the general structure I above can be prepared in a variety of ways, for example:

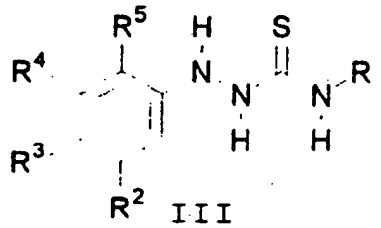
a) treating thiohydrazides of general structure II, or the corresponding thiohydrazones, in hot acetic acid in air,



II

; or

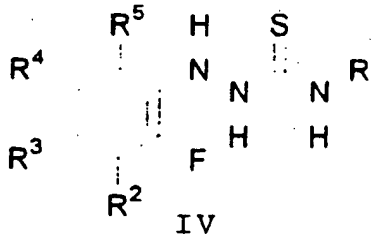
b) reacting compounds of the general structure III with bromine,



III

; or

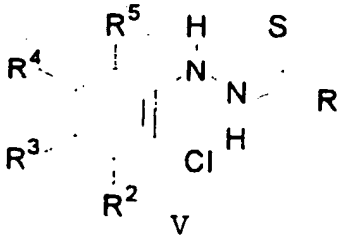
c) heating compounds of general structure IV in a protic solvent,



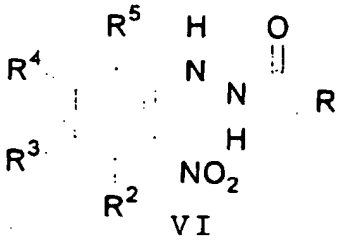
IV

; or

d) reacting compounds of the general structures V or VI with sodium hydride,



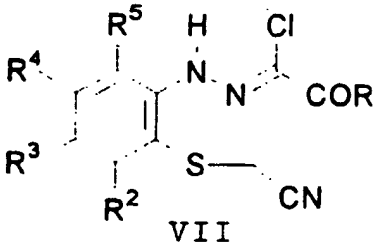
V



VI

; or

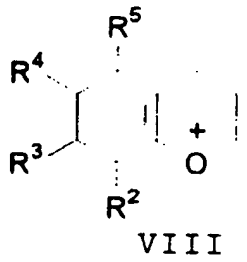
e) reacting compounds of the general structure VII with a base,



VII

; or

f) reacting pyrylium compounds of general structure VIII with an appropriate nucleophile,

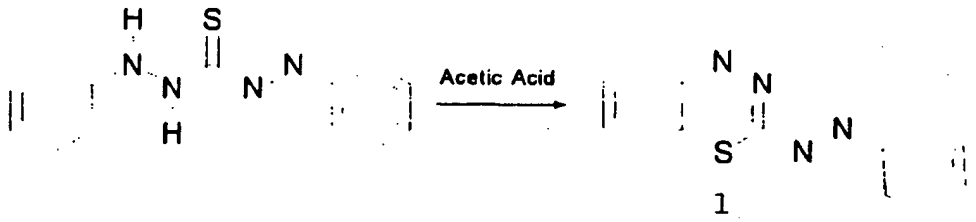


VIII

where R^2, R^3, R^4, R^5, are as defined above and R is taken from the group: Ar, NHAr, NHNHAr, COAr, carboalkoxy, alkoxy, NR^6COAr, CH_2OAr, and CH_2NR^6Ar, in which Ar and R^6 are as described above, followed, optionally, by conversion of any one or more of the groups, R, R^2, R^3, R^4, R^5 into new groups R, R^2, R^3, R^4, R^5 by deprotection, coupling, addition, substitution, or elimination; or by oxidation of the sulfur to sulfoxide or sulfone; and, if desired, by converting a compound of the general structure I into its salt or setting it free from its salt.

Example:

Diphenyl thiohydrazone is heated in refluxing acetic acid in air for 30 to 90 minutes to afford benzothiadiazene **1**.



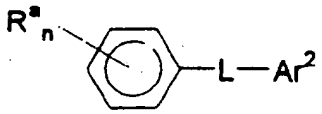
1

Specific representatives of compounds of the general structure I include:

3-phenylazo-1H-4,1,2-benzothiadizine

2-phenylazo-2H-benzopyran

Another group of compounds suitable for use in the methods of the invention are compounds of th formula:



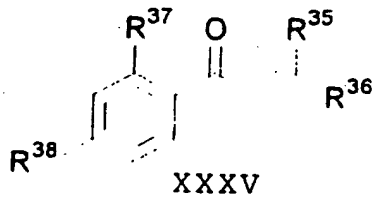
wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a flexible linker which does not contain nitrogen; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

Particularly preferred embodiments of this group of compounds are those of the formula:



XXXV

in which

R^{35} is taken from the group: H, hydroxy, alkoxyl, acyloxy, and silyloxy;

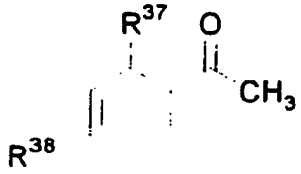
R^{36} is either Ar, or COAr, in which Ar is (un)substituted phenyl in which the allowed substituents are taken from the group: H, hydroxy, (un)substituted alkoxy, acyloxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, or (un)substituted alkynyl, carboxy, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl, and aryl;

R^{37} is taken from the group: H, hydroxy, alkoxy, halo, acyloxy, and siloxyl;

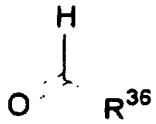
R^{38} is taken from the group: H, hydroxy, alkoxy, acyloxy, siloxy, (un)substituted alkoxy, acyloxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, and (un)substituted alkynyl,

or salts thereof.

Compounds of general structure XXXV can be prepared by treating an acetophenone of general structure XXXVI with an appropriate aldehyde of general structure XXXVII under either basic or acidic conditions,

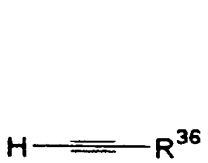


XXXVI

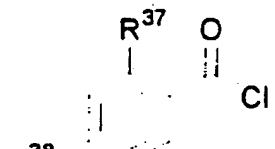


XXXVII

or by treating an appropriate alkyne of general structure XXXVIII with an acid halide of general structure XXXIX in the presence of a suitable catalyst, such as aluminum trichloride,

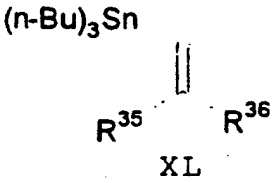


XXXVIII



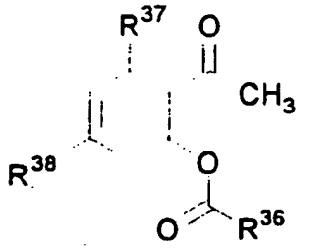
XXXIX

or by treating acid halides of the general structure XXXIX with (E)-1,2-bis(tri-n-butylstanyl)ethylene, or with a vinylstanane of general structure XL in the presence of a suitable catalyst, for example, a palladium catalyst.



XL

or by treating an acetophenone of general structure XLI with a strong base,



XLI

where R^{35}, R^{36}, R^{37}, and R^{38} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{35}, R^{36}, R^{37}, and R^{38} into new groups R^{35}, R^{36}, R^{37}, and R^{38} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XXXV into its salt or setting it free from its salt.

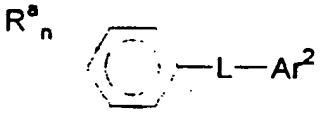
Specific representative compounds of general structure XXXV include:

2,4-dimethoxy-2'-hydroxychalcone

1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)propan-1,3-dione

1,4-dioxo-1,4-diphenylbut-2-ene

Still another group of compounds useful in the invention are those of the formula:



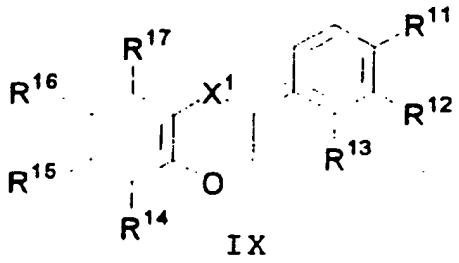
wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

Particularly preferred compounds in this group are those of formulas IX, XIV, and XX as follows:



IX

in which:

R^{11} and R^{12} are individually taken from the group:

H, hydroxy, C_{1-6} alkoxy, acetyloxy, and C_{1-12} (un)substituted alkyl;

R^{13}, R^{14} and R^{17} are individually taken from the group:

H, hydroxy, C_{1-6} straight or branched chain alkoxy, and acetyloxy;

R^{15} is taken from the group: Hydroxy, (un)substituted C_{1-12} alkoxy, C_{1-12} alkyl, (un)substituted alkenyl, and acetyloxy;

R^{16} is taken from the group: H, hydroxy, (un)substituted lower alkoxy, acetoxy, (un)substituted alkyl, and (un)substituted alkenyl;

where R^{11}, R^{12} may form a 5-7 member (un)substituted carbocycle or heterocycle;

where R^{15}, R^{16} may form a 5-7 member (un)substituted carbocyclic or heterocyclic ring;

X^1 is either carbonyl or CH_2;

and the dotted line may be a double bond,

in which permissible substituents on the above mentioned substituted groups include: Lower alkyl, lower alkoxyl, hydroxy, siloxy, halo, carboxyl, and aryl, with the following provisions:

if X^1 is carbonyl and

if R^{15} is hydroxy and if only one of R^{11}, R^{12}, or R^{13} is hydroxy, then at least one of R^{14}, R^{16}, and R^{17} must be other than H;

or if R^{15} is alkoxy, and if R^{11}, R^{12}, R^{13} together are H, then R^{17} can be neither H nor hydroxy;

or if R^{15} is (un)substituted alkoxy, and if R^{11}, R^{12}, and R^{13} together consist of only H, or H and one or two alkoxy, and R^{17} is H, then R^{14} must be other than H, Me or hydroxymethyl;

or if R^{15} is hydroxy or alkoxy, and if R^{11}, R^{12}, R^{13} together consist of only H, or H and one or two alkoxy, or H and only one or two alkyl, and R^{17} is C_{1-4} alkyl, then at least one of R^{14} and R^{16} must be other than H;

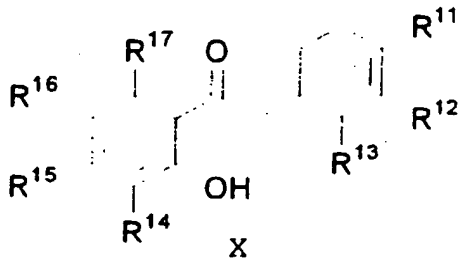
or if R^{15} is hydroxy and if R^{11}, R^{12}, R^{13}, R^{14}, and R^{16} all are H, R^{17} must be neither H nor hydroxy;

or if R^{15} is iso-propoxy, and if R^{11}, R^{12}, and R^{13} together consist of only H, or H and one or two hydroxys, then at least one of R^{14}, R^{16}, R^{17} must be other than H;

or if R^{15} is 1,5 di(lower) alkyl C_{5-10} alkyl, then at least one of R^{11}, R^{12}, R^{13}, R^{14}, R^{16}, and R^{17} must be other than H;

or salts thereof.

Compounds of the general structure shown above can be made by a process wherein ketones of the structure (X) shown below:



are reacted with an alkylorthoformate in the presence of a base, or

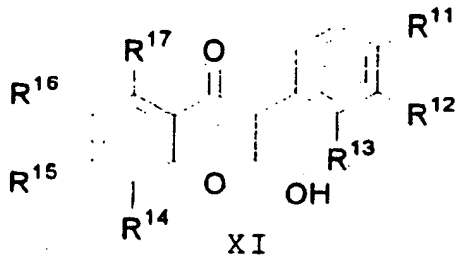
are reacted with an ethyloxalyl chloride in the presence of pyridine, followed by hydrolysis and decarboxylation, or

are reacted with an alkyl formate in the presence of an alkali metal, or

are reacted with an N,N-dialkyl formamide in the presence of phosphorous oxychloride, or

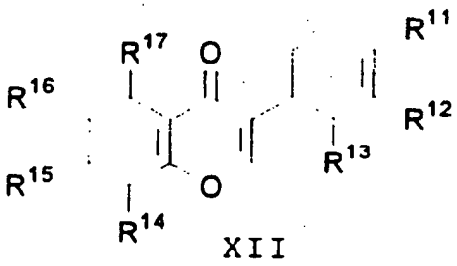
are reacted with a cyanide in the presence of hydrogen halide,

or by dehydrating 2-hydroxyisoflavanoids of the general structure (XI):



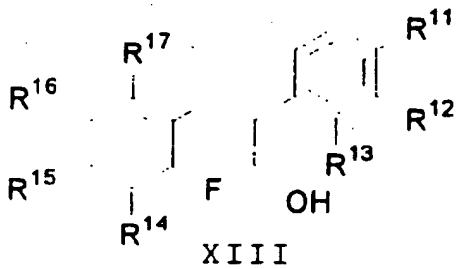
XI

or by subjecting compounds of the general structure XII to catalytic hydrogenation,



XII

or by treating compounds of the general structure XIII, available from alkylation of the corresponding phenylacetate with an appropriate benzylhalide, followed by reduction, with $(PF_6)_2Rh(EtC_5Me_4)(C_6H_6)$,



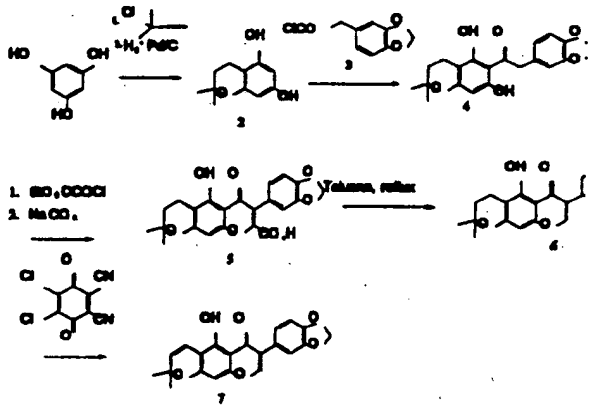
XIII

in which the groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} are as defined above, followed, optionally, by the conversion of any one or more of groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} into new groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} by deprotection,

dehydrogenation, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure IX into its salt or setting it free from its salt.

Example:

1,3,5-trihydroxybenzene is allowed to react with iso-pentynyl chloride, followed by catalytic hydrogenation, to give product **2**. The compound **2** is allowed to react with the acid chloride **3** to provide the ketone **4**. Ketone **4** is treated with ethyloxalyl chloride in pyridine at 0°C to afford an ester, which is hydrolyzed in aqueous acetone containing sodium carbonate to give the acid **5**. When heated in refluxing toluene, acid **5** undergoes decarboxylation to give compound **6**, which upon treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gives the isoflavanoid **7**.



Specific representatives of compounds of the general structure IX include:

tectorigenin

robustone

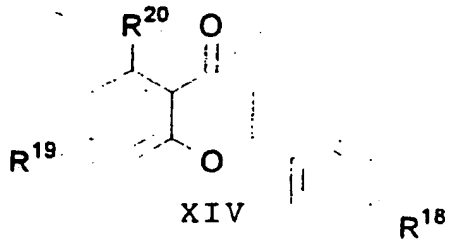
robustone methyl ether

7,2',4'-trihydroxyisoflavone

6,2',3'-trihydroxy-7,4'-dimethoxyisoflavan

8,4'-dimethoxy-7-hydroxisoflavone

Comp unds of XIV have the structure:



XIV

in which:

R^{18} and R^{19} are individually taken from the group:

H, hydroxy, (un)substituted alkyl, (un)substituted alkoxy, COR^{21} carboxy, carboalkoxy, OR^{22}, carbamoyl optionally substituted at the nitrogen by lower chain alkyl or phenyl, acyloxy, halo, cyano, and azido.

R^{20} is taken from the group: H, hydroxy, halo, lower chain alkyl, acyloxy, and siloxy;

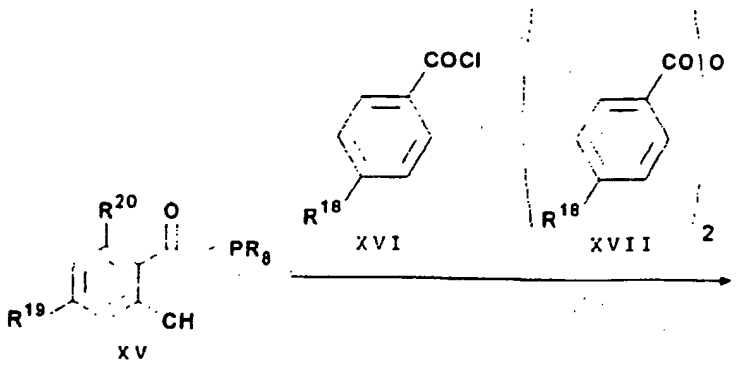
in which R^{21} is taken from the group: Alkyl, alkenyl,

alkynyl, aralkyl, (un)substituted phenyl, (un)substituted naphthyl, thienyl, furanyl, and pyridyl;

and R^{22} is comprised of a C_{3-6} carbohydrate moiety;

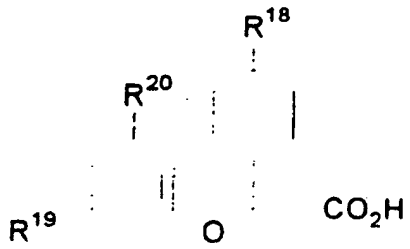
or salts thereof.

Compounds of general structure XIV can be prepared by reacting ylides of general structure XV with either acid chlorides of general structure XVI or acid anhydrides of general structure XVII:



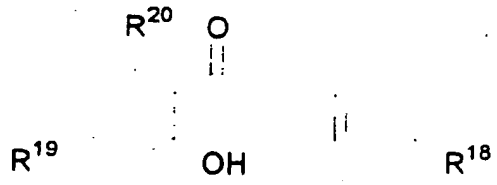
XV XVI XVII

or by treating acids of the general structure XVIII with polyphosphoric acid, trifluoracetic anhydride, or similar reagent,



XVIII

or by treating chalcones of general structure XIX with either base, or with base followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.



XIX

in which the groups R^{18}, R^{19}, R^{20} are as above, followed, optionally, by conversion of any one or more of the groups R^{18}, R^{19}, R^{20} into new groups R^{18}, R^{19}, R^{20} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of general structure XIV into its salt or setting it free from its salt.

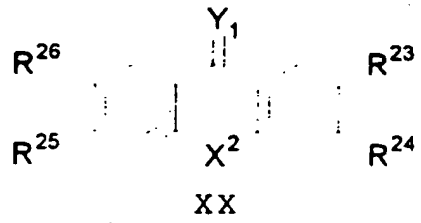
Specific representatives of compounds of the general structure XIV are:

5,4'-dimethyl-7-acetylflavone

7-benzoyloxyflavanone

apiin acetate

Compounds of structure XX are of the formula:



XX

where

R^{23}, R^{24}, R^{25}, R^{26} are individually taken from the group:

H, hydroxy, (un)substituted alkoxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, halo, carboxyl, and acyloxy, and where R^{23} and R^{24}, and likewise R^{25} and R^{26}, can together equal a 5-7 member (un)substituted carbocyclic or heterocyclic ring, and where substituents on the above mentioned optionally substituted groups may include lower chain alkyl, lower chain alkoxy, hydroxy, siloxy, acyloxy, halo, benzoyl, carboxy, carboalkoxy, and carbamoyl optionally substituted at nitrogen with lower chain alkyl, phenyl, thienyl, furyl, or pyridinyl;

Y^1 is taken from the group: O, $-OCH_2CH_2O-$, $-OCH_2CH_2S-$, $-OCH_2CH_2CH_2O-$, $-SCH_2CH_2CH_2S-$, and $-SCH_2CH_2S-$;

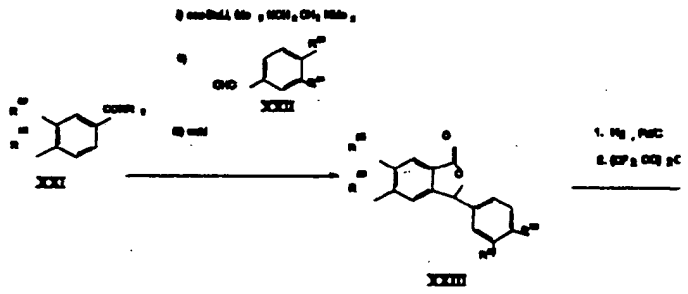
X^2 is taken from the group: CH_2, O, and S;

with the following provisions:

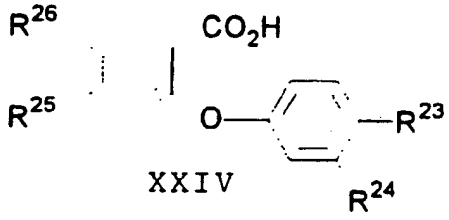
if X and Y are O and R^{24} or R^{25} are either both alkoxy, or alkoxy and alkyl, irrespective of order, then at least one of R^{23} and R^{26} must be other than H,

or salts thereof.

Compounds of the general structure XX can be prepared by reacting amides of general structure XXI with *sec*-butyl lithium and tetramethylethylenediamine in THF, followed by addition of benzaldehydes of general structure XXII, and the addition of acid. The resulting lactones of general structure XXIII can be reduced by catalytic hydrogenation or treatment with activated zinc in acid, followed by dehydration with trifluoracetic anhydride,



or, by treating diaryl ethers of general structure XXIV with sulfuric acid, alumium trichloride, trifluoracetic anhydride, or similar reagent,

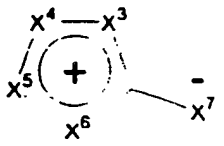


where R^{23}, R^{24}, R^{25}, R^{26} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{23}, R^{24}, R^{25}, R^{26} into new groups R^{23}, R^{24}, R^{25}, R^{26} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XX into its salt or setting it free from its salt.

Specific representatives of compounds of the general structure XX include:

3-isopropoxyanthrone

Another group that is useful in the invention are of the formula:



XXV

in which:

X^3 is NR^{27}, X^4 is CR^{30}, X^5 is O, X^6 is CR^{31}, X^7 is O^-;

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{31}, X^6 is CR^{28}, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is NR^{28}, X^6 is CR^{31}, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{28} or N, X^5 is NR^{30}, X^6 is CR^{29}, X^7 is NR^{32};

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{28}, X^6 is CR^{29}, X^7 is NR^{32};

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is S, X^6 is CR^{31}, X^7 is NR^{32};

or X^3 is NR^{30}, X^4 is CR^{27}, X^5 is S, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is CR^{31}, X^7 is O^- or S^-;

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{27}, X^5 is NR^{30}, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is S, X^6 is CR^{27}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is S, X^6 is CR^{31}, X^7 is O^-;

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{31}, X^6 is N, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is NR^{28}, X^4 is N, X^7 is NR^{32} or CZ^2Z^3;

or X^3 is NR^{27}, X^4 is CR^{28} or N, X^5 is NR^{30}, X^4 is N, X^7 is NR^{32} or CZ^2Z^3;

or X^3 is NR^{30}, X^4 is N, X^5 is S, X^6 is CR^{31}, X^7 is O^-;

or X^3 is S, X^4 is CR^{27}, X^5 is NR^{30}, X^6 is N, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is N, X^7 is NR^{32};

or X^3 is O or S, X^4 is N, X^5 is NR^{30}, X^6 is N, X^7 is NR^{32};

in which

R^{27}, R^{28} and R^{29} are individually straight or branched chain lower alkyl;

R^{30} and R^{31} are individually taken from the group:

hydrogen, straight or branched chain (un)substituted alkyl, (un)substituted aromatic, in which the substituents may include: Halogen, straight or branched chain lower alkyl, alkenyl, alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, straight or branched chain alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy;

R^{32} is taken from the group:

Ar, COAr, COR^{33}, where Ar is a six-membered (un)substituted aromatic ring, in which substituents on this ring may include: Halogen, straight or branched chain lower alkyl, alkenyl, alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, straight or branched chain alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy;

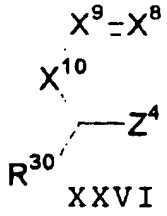
R^{33} is taken from the group: Hydrogen, and straight or branched chain alkyl;

Z^2 and Z^3 are individually taken from the group: CN and CO_2R^{34};

R^{34} is taken from the group: Hydrogen, straight or branched chain alkyl, and (un)substituted aromatic;

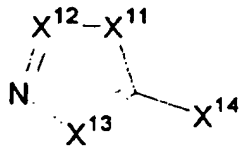
or salts thereof.

Compounds of general structure XXV above can be prepared by treating compounds of general structure XXVI, where X^8 is NR^{30} or S, X^9 is CR^{30} or N, X^{10} is NR^{30} or S, Z^4 is CO_2H, CO_2R^{30} or CN, with acid chlorides or anhydrides,



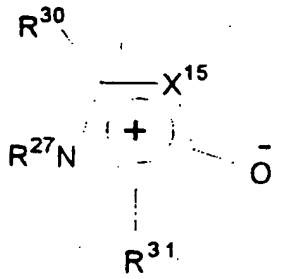
XXVI

or by reacting compounds of general structure XXVII, where X^{11} is NR^{30} or S, X^{12} is N or CR^{30}, X^{13} is hal gen, SMe, or OEt, with amines, sulfides or enolates.



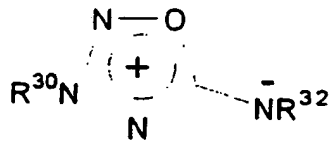
XXVII

or by reacting compounds of general structure XXVIII, where X^{15} is O or S with isocyanates, isothiocyanates, or carbon disulfide.



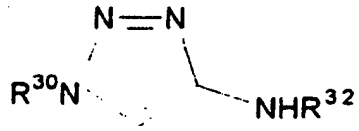
XXVIII

or by reacting compounds of general structure XXIX with sodium hydroxide,



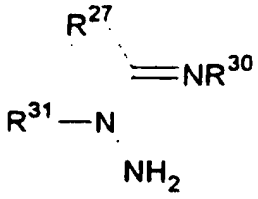
XXIX

or by reacting compounds of general structure XXX with alkyl tosylates, aryl tosylates or alkyl halides,



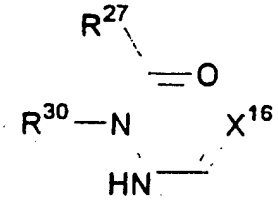
XXX

or by reacting compounds of general structure XXXI with aryl isocyanide dichlorides, phosgene, thiophosgene, or 3,3-bis(methylthio)acrylonitriles,



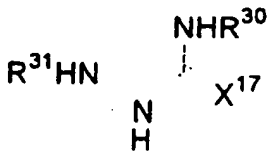
XXXI

or by reacting compounds of general structure XXXII, where X^{16} is O, S, or NH, with sodium ethoxide or HCl in the presence of acid chlorides or HCl in the presence of acid anhydrides,



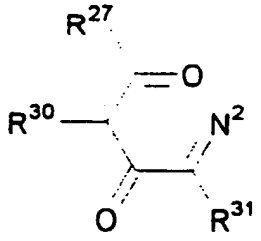
XXXII

or by reacting compounds of general structure XXXIII, where X^{17} is NH or S, with acid chlorides, acid anhydrides, or HONO,



XXXIII

or by reacting compounds of general structure XXXIV with $Cu(acac)_2$,



XXXIV

where R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} into new groups R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XXV into its salt or setting it free from its salt.

Specific representatives of compounds of the general structure XXV include:

3-(4-chlorophenyl)-1,2,3,4-oxatriazolium-5-(4-chlorophenyl)aminide

1,3-di(4-methylphenyl)-1,2,3,4-tetrazolium-5-oxide.

The following examples are intended to illustrate, but not to limit, the invention.

Example 1

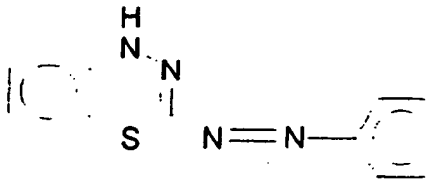
Compound 59-0008 was synthesized according to the procedure of McDonald, W. S., *et al. Chem Comm* (1969) 392-393; Irving, H. N. N. H. *et al. Anal Chim Acta* (1970) 49:261-266. Briefly, 10.0 g of dithizone was taken up in 100 ml EtOH and 50 ml AcOH and heated at reflux for 18 h. After cooling, this was diluted first with 100 ml water and then with 50 ml 1N NaOH. This was then further neutralized by the addition of 6 N NaOH to bring the pH to 5.0. This deep purple mixture was then concentrated on a rotavapor to remove organics. Once the liquid had lost all of its purple color, this was filtered to collect the dark precipitate. Purification by flash chromatography (4.5 x 25.7 cm; EtAc/Hep. (1:4); Rf 0.22) followed by recrystalization from EtOH gave 2.15 g (25% yield) of dark

purple crystals, mp=184-185 °C. ^{1}H NMR ($CDCl_3$) 7.90 (d of d, J_1=7.7, J_2=2.2, 2H), 7.64 (hump, 1H), 7.49 (m, 3H), 7.02 (m, 1H), 6.91 (m, 2H), 6.55 (d, J=8.1, 1H). MS (EI) 254 (47, M+), 105 (26), 77 [100], 51 (27). HRMS (EI, M+) 254.0626 (calcd 254.0626182). Anal. Calcd for $C_{13}H_{10}N_4S$: C, 61.40; H, 3.96; N, 22.03. Found: C, 61.40; H, 4.20; N, 22.06.

Example 2

High Throughput Screening

Several thousand compounds were tested in the assay system set forth in U.S. Serial No. 08/458,434, filed 2 June 1995, and incorporated herein by reference. The standard positive control was a compound of the invention, 59-0008 (also denoted "OS8"), which is of the formula:



"OS8"

In more detail, the 2T3-BMP-2-LUC cells, a stably transformed osteoblast cell line described in Ghosh-Choudhury *et al. Endocrinology* (1996) 137:331-39, referenced above, was employed. The cells were cultured using α-MEM, 10% FCS with 1% penicillin/streptomycin and 1% glutamine ("plating medium"), and were split 1:5 once per week. For the assay, the cells were resuspended in a plating medium containing 4% FCS, plated in microtiter plates at a concentration of 5 x 10^3 cells (in 50 µl)/well, and incubated for 24 hours at 37°C in 5% CO_2. To initiate the assay, 50 µl of the test compound or the control in DMSO was added at 2X concentration to each well, so that the final volume was 100 µl. The final serum concentration was 2% FCS, and the final DMSO concentration was 1%. Compound 59-0008 (10 µM) was used as a positive control.

The treated cells were incubated for 24 hours at 37°C and 5% CO_2. The medium was then removed, and the cells were rinsed three times with PBS. After removal of excess PBS, 25 µl of 1X cell culture lysing reagent (Promega #E153A) was added to each well and incubated for at least ten minutes. Optionally, the plates/samples could be frozen at

this point. To each well was added 50 µl of luciferase substrate (Pr mega #E152A; 10 ml Promega luciferase assay buffer per 7 mg Promega luciferase assay substrate). Luminescence was measured on an automated 96-well luminometer, and was expressed as either picograms of luciferase activity per well or as picograms of luciferase activity per microgram of protein.

In this assay, compound 59-0008 (3-phenylazo-1H-4,1,2-benzothiadiazine) exhibited a pattern of reactivity, as shown in Figure 1. The activity for compound 59-0008 was maximal at a concentration of approximately 3-10 µM and, more particularly, at about 3 µM, and thus provided a response of approximately 175 light emission units. Accordingly, other tested compounds were evaluated at various concentrations, and these results were compared to the results obtained for 59-0008 at 10 µM (which value was normalized to 100). For instance, any tested compound in Figure 2 and Figure 3 that showed greater activity than 10 µM of 59-0008 would result in a value over 100.

As shown in Figure 2 (39 sheets) and Figure 3 (10 sheets), several compounds were found to be particularly effective.

Example 3

In vivo Calvarial Bone Growth Data

Compound 59-0008 was assayed *in vivo* according to the procedure described previously (see "*In vivo* Assay of Effects of Compounds on Murine Calvarial Bone Growth", *supra*). As compared to a vehicle control, compound 59-0008 induced a 4-fold increase in width of new calvarial bone.

Example 4

Chondrogenic Activity

Compounds 59-008, 59-0102 and 50-0197 were assayed for effects on the differentiation of cartilage cells, as compared to the action of recombinant human BMP-2. Briefly, a mouse clonal chondrogenic cell line, TMC-23, was isolated and cloned from costal cartilage of transgenic mice containing the BMP-2 gene control region driving SV-40 large T-antigen, generated as described in Ghosh-Choudhury *et al Endocrinology* 137:331-39, 1996. These cells were cultured in DMEM/10% FCS, and were shown t

express T-antigen, and also to produce aggrecan (t luidine blue staining at pH 1.0) and Type-II collagen (immunostaining) by 7 days after confluence.

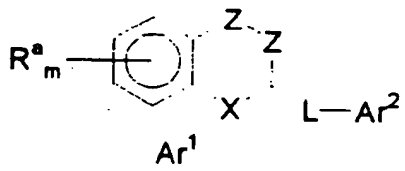
For measurement of alkaline phosphatase (ALP) activity, the technique of LF Bonewald *et al. J Biol Chem* (1992) 267:8943-49, was employed. Briefly, TMC-23 cells were plated in 96 well microtiter plates in DMEM containing 10% FCS at 4 x 10^3 cells/well. Two days after plating, the cells were confluent and the medium was replaced with fresh medium containing 10% FCS and different concentrations of compounds or recombinant BMP-2. After an additional 2 or 5 days incubation, the plates were washed twice with PBS, and then lysing solution (0.05% Triton X-100) was added (100 µl/well). The cells were lysed by three freeze-thaw cycles of -70°C (30 min), followed by 37°C (30 min with shaking). Twenty microliters of cell lysates were assayed with 80 µl of 5 mM p-nitrophenol phosphate in 1.5 M 2-amino-2-methyl-propanol buffer, pH 10.3 (Sigma ALP kit, Sigma Chemical Co., St. Louis, MO) for 10 min at 37°C. The reaction was stopped by the addition of 100 µl of 0.5 M NaOH. The spectrophotometric absorbance at 405 nm was compared to that of p-nitrophenol standards to estimate ALP activity in the samples. The protein content of the cell lysates was determined by the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Specific activity was calculated using these two parameters.

At day 2, compounds 59-0008 (10^{-9} M), 59-0102 (10^{-7} M) and 59-0197 (10^{-9} M) increased ALP levels approximately 3-, 2- and 2.5-fold, respectively, as compared to the vehicle control. Recombinant BMP2 at 100, 50 or 10 ng/ml induced ALP levels approximately 10-, 4- or 1.5-fold, respectively, as compared to the vehicle control.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

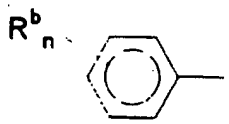
L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

2. The method of claim 1 wherein L is a flexible conjugated linker.

3. The method of claim 1 wherein L is selected from the group consisting of a covalent bond, -N=N-, -RC=CR-, -RC=N-, -N=CR-, -NRCO-, -CONR-, $-CR_2O-$, and $-CR_2NR-$ where each R is independently H or alkyl (1-6C).

4. The method of claim 1 wherein Ar^2 is



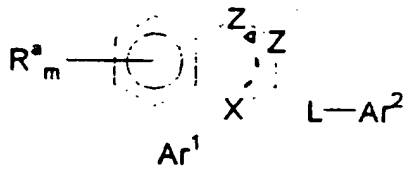
where R^b is a non-interfering substituent and n is an integer from 0 to 5.

5. The method of claim 4 wherein Ar^2 is unsubstituted phenyl.

6. The method of claim 1 wherein said compound is 59-0008.

7. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

8. The composition of claim 7 wherein L is a flexible conjugated linker.

9. The composition of claim 7 wherein L is selected from the group consisting of a covalent bond, -N=N-, -RC=CR-, -RC=N-, -N=CR-, -NRCO-, -CONR-, $-CR_2O$, and $-CR_2NR$- where each R is independently H or alkyl (1-6C).

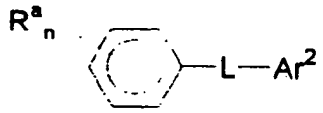
10. The composition of claim 7 wherein Ar^2 is

where R^b is a non-interfering substituent and n is an integer from 0 to 5.

11. The composition of claim 7 wherein Ar^2 is unsubstituted phenyl.

12. The composition of claim 7 wherein said compound is 59-0008.

13. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;
n is an integer of 0 and 5;
L is a flexible linker which does not contain nitrogen; and
Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

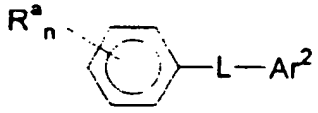
14. The method of claim 13 wherein R^a is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

15. The method of claim 13 wherein Ar^2 is substituted or unsubstituted phenyl.

16. The method of claim 13 wherein Ar^1 and Ar^2 are different.

17. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a flexible linker which does not contain nitrogen; and

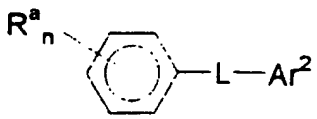
Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

18. The composition of claim 17 wherein R^a is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

19. The composition of claim 17 wherein Ar^2 is substituted or unsubstituted phenyl.

20. The composition of claim 17 wherein Ar^1 and Ar^2 are different.

21. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

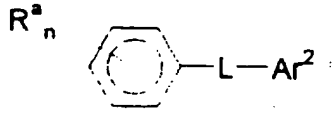
Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

22. The method of claim 21 wherein R^1 is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

23. The method of claim 21 wherein Ar^2 is substituted or unsubstituted phenyl.

24. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

25. The composition of claim 24 wherein R^1 is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

26. The composition of claim 24 wherein Ar^2 is substituted or unsubstituted phenyl.

27. The method of any of claims 1, 13 or 21 wherein said condition is osteoporosis, bone fracture or deficiency, primary or secondary hyperparathyroidism, periodontal disease or defect, metastatic bone disease, osteolytic bone disease, post-plastic surgery, post-prosthetic joint surgery, or post-dental implantation.

28. The method of any of claims 1, 13 or 21 which further comprises administering to said subject one or more agents that promote bone growth or that inhibit bone resorption.

29. The method of claim 28 wherein said agents are selected from the group consisting of bone morphogenetic factors, anti-resorptive agents, osteogenic factors, cartilage-derived morphogenic proteins, growth hormones, and differentiating factors.

2TJL-C9 CELLS			10/1/96				
5x 10^3 Cells/well							
	uM	READ 1	READ 2	AVERAGE	INDUCTION	AVE-BASAL	% MAX
OS-8	100.000	0.21	0.22	0.22	0.18	-0.99	-17.80
	31.250	3.96	4.44	4.20	3.49	3.00	54.26
	9.766	6.99	6.46	6.72	5.59	5.52	100.00
	3.052	4.62	4.88	4.75	3.95	3.55	64.22
	0.954	3.13	3.16	3.14	2.61	1.94	35.12
	0.298	2.75	2.59	2.67	2.22	1.47	26.58
	0.093	2.10	2.04	2.07	1.72	0.87	15.77
	0.029	1.56	1.71	1.63	1.36	0.43	7.80
	0.0091	1.45	1.42	1.44	1.19	0.23	4.21
	0.0028	1.28	1.37	1.33	1.10	0.12	2.25
	0.0000	1.32	1.30	1.31			
	0.0000	1.20	1.00	1.10			
		AVERAGE BASAL		1.20			

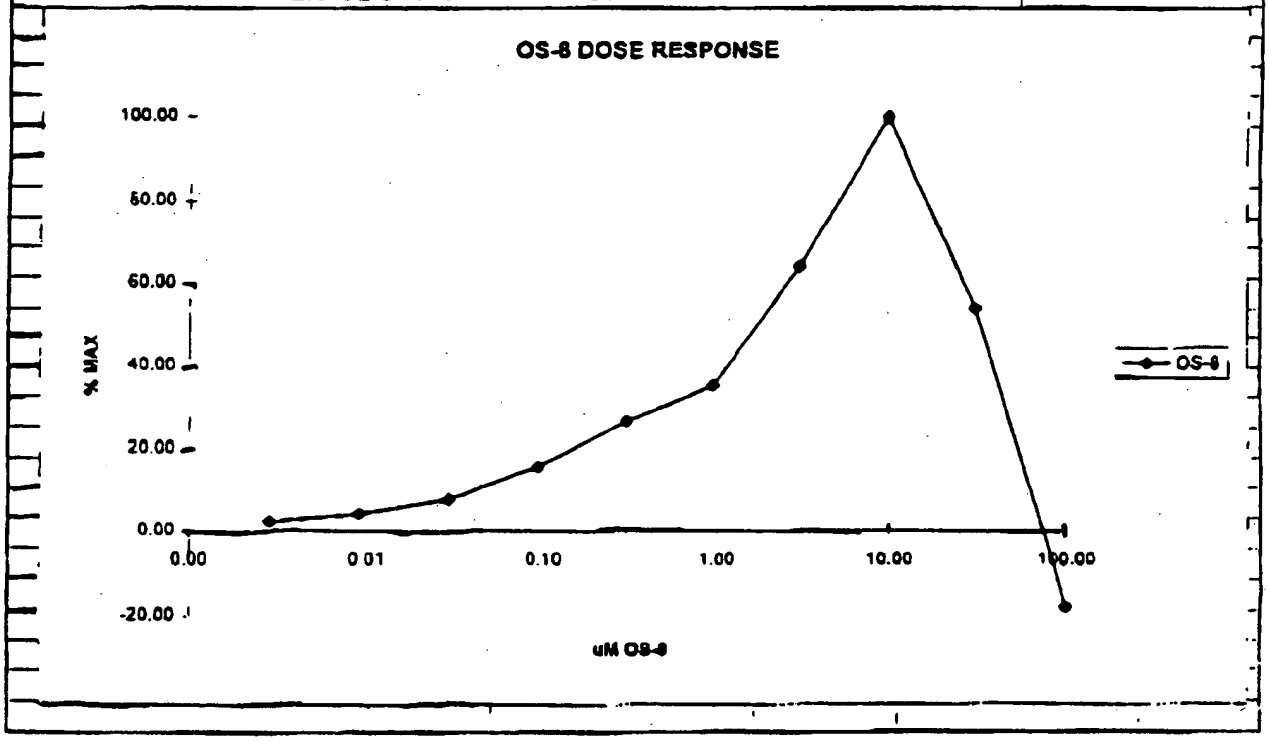


Figure 1

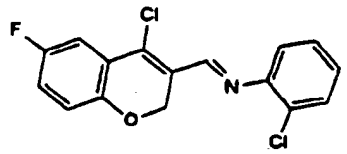
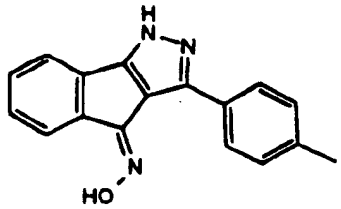
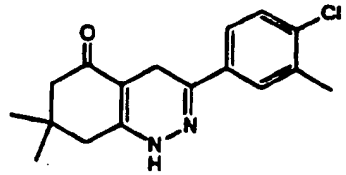
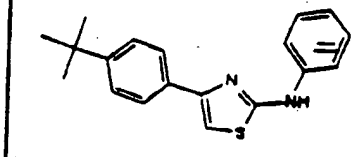
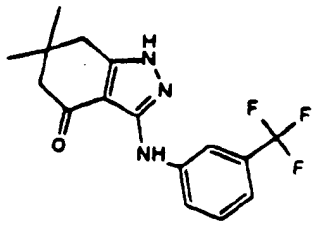
	21.433		69.74
233.289	4.287		31.59
	0.857		39.70
	0.171		18.29
 92-6353			
92-6353	155.199	uM	
	31.040		204.14
322.166	15.520		154.94
	3.104		28.09
	1.552		
	0.310		3.53
 92-8007			
92-8007	161.613	uM	-16.65
	36.323		58.65
275.311	18.161		142.33
	3.632		45.65
	1.816		
	0.363		4.47
 92-8215			
92-8215	165.123	uM	32.90
	33.025		151.08
302.805	16.512		132.29
	3.302		59.90
	1.651		
	0.330		23.34

Figure 3



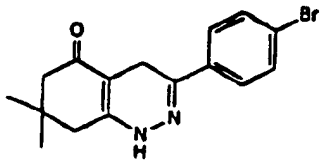
92-8258

92-8258			
	162.102	uM	-16.65
	32.420		157.44
308.447	16.210		101.04
	3.242		39.02
	1.621		
	0.324		12.78



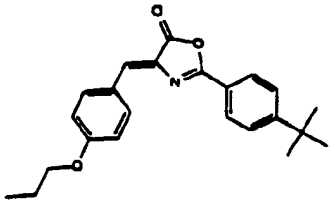
92-8362

92-8362			
	154.647	uM	136.79
	30.929		137.00
323.318	15.465		65.02
	3.093		17.34
	1.546		
	0.309		0.41

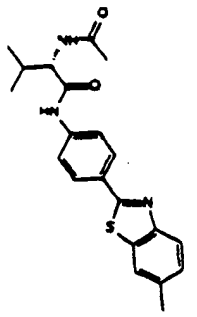


92-8372

92-8372			
	150.045	uM	63.76
	30.009		134.71
333.234	15.004		92.08
	3.001		31.35
	1.500		
	0.300		13.20

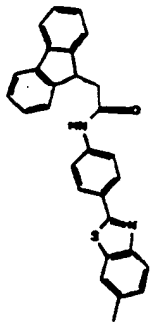


92-9183



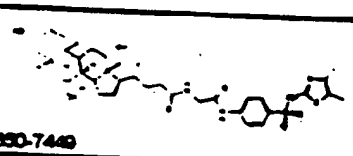
650-7377			
650-7377			
		131.062	uM
		13.106	
	381.466	2.621	
		0.524	
		0.105	

	-50.32
	68.27
	116.61
	61.26
	35.86



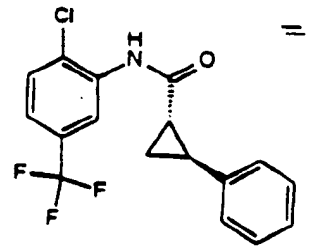
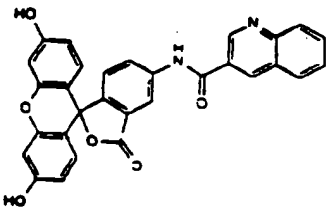
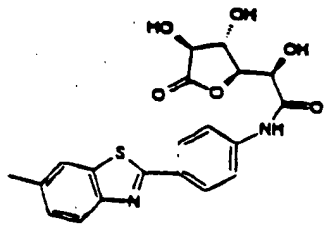
650-7413			
650-7413			
		111.964	uM
		11.196	
	446.572	2.239	
		0.448	
		0.090	

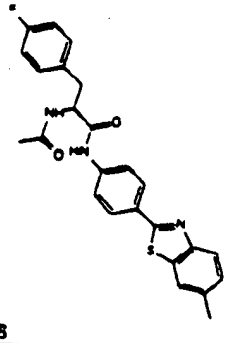
	-40.44
	-2.55
	157.01
	78.73
	23.91



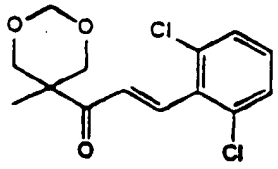
650-7449			
650-7449			
		69.938	uM
		6.994	
	714.923	1.399	
		0.280	
		0.056	

	-42.42
	73.79
	112.16
	75.24
	28.38

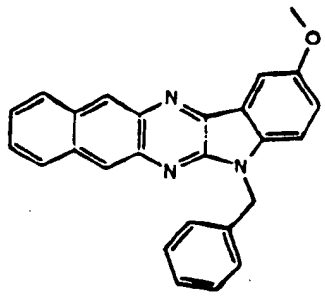
 =			
650-9287			
650-9287		147.170	uM
		14.717	
	339.744	2.943	
		0.589	
		0.118	
			-15.82
			15.82
			130.71
			91.11
			69.05
			
650-9356			
650-9356		99.506	uM
		9.951	
	502.462	1.990	
		0.398	
		0.080	
			-24.650	
			83.140	
			168.810	
			45.470	
			9.740	
			
650-9467			
650-9467		120.646	uM
		12.065	
	414.436	2.413	
		0.483	
		0.097	
			-19.800	
			112.990	
			122.730	
			43.520	
			33.140	



650-9576			
650-9576		111.724	uM
		11.172	
	447.532	2.234	
		0.447	
		0.089	

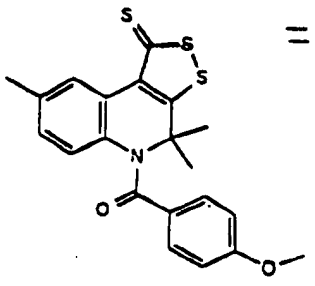


695-0262			
695-0262		166.019	uM
		33.204	
	301.169	16.602	
		3.320	
		0.332	

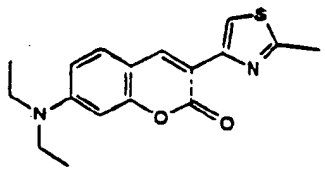


695-0268			
695-0268		128.363	uM
		25.677	
	389.459	12.839	
		2.568	
		0.257	

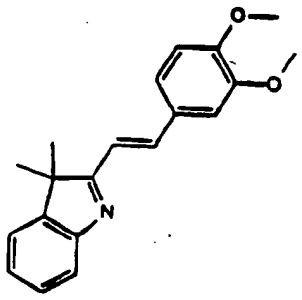
	-27.430
	90.660
	101.610
	44.900
	19.930
	-19.16
	-12.60
	148.26
	-2.23
	-3.07
	-16.87
	40.25
	169.96
	195.29
	14.02



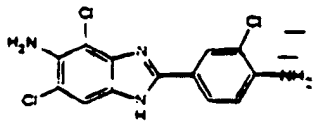
895-0594				
895-0594				
		120.896	uM	-21.63
		12.090		25.89
	413.58	2.418		122.10
		0.484		75.32
		0.097		39.42



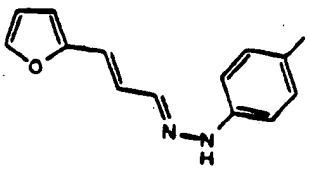
895-0857				
895-0857				
		159.028	uM	-30.48
		15.903		146.74
	314.407	3.181		74.54
		0.636		25.82
		0.127		3.66



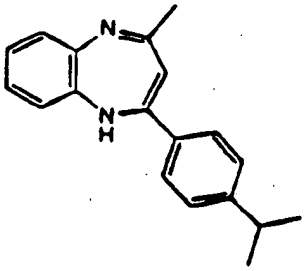
895-0084				
895-0084				
		162.655	uM	-31.06
		16.265		325.06
	307.393	3.253		87.51
		0.651		40.39
		0.130		16.03



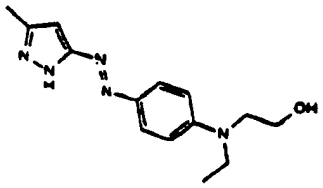
695-1161		
695-1161	152.625	uM
	15.263	
327.602	3.053	
	0.611	
	0.122	



695-1420		
695-1420	220.965	uM
	22.097	
226.279	4.419	
	0.884	
	0.177	

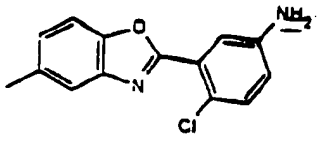


695-1679		
695-1679	180.910	uM
	18.091	
276.383	3.618	
	0.724	
	0.145	

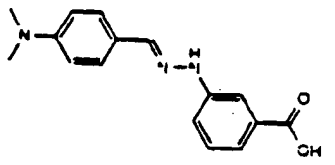


695-1691		
695-1691	182.922	uM
	18.292	
273.34	3.658	

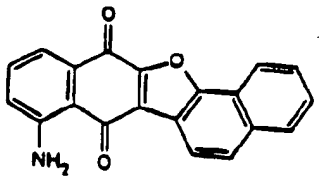
-5.51	
109.31	
56.06	
29.49	
24.71	
-19.47	
110.50	
49.64	
33.65	
20.06	
-30.36	
111.72	
102.83	
18.01	
0.44	
-16.29	
90.84	
105.70	



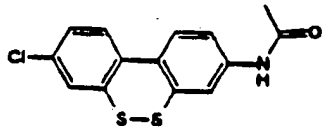
695-3846			
695-3846		193.267	uM
		19.327	
	258.708	3.865	
		0.773	
		0.155	
			-21.41
			13.40
			114.46
			52.12
			38.29



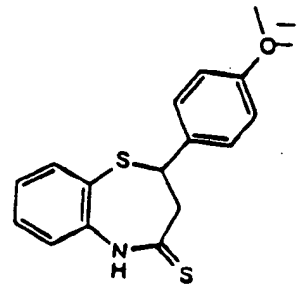
695-4642			
695-4642		176.473	uM
		17.647	
	283.331	3.529	
		0.706	
		0.141	
			6.97
			283.89
			447.51
			304.86
			100.45



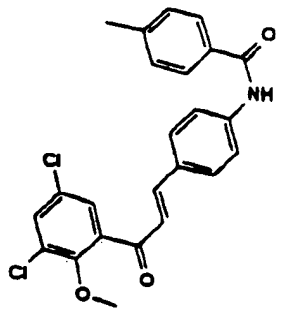
695-4643			
695-4643		159.581	uM
		15.958	
	313.312	3.192	
		0.638	
		0.128	
			-17.18
			24.64
			100.12
			60.37
			27.85



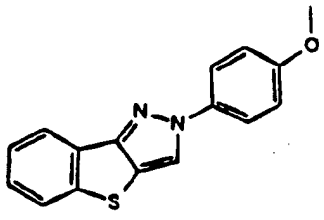
695-5185			
695-5185		162.433	uM
		16.243	
	307.821	3.249	
		0.650	
		0.130	
			-6.47
			213.42
			107.63
			46.75
			18.27



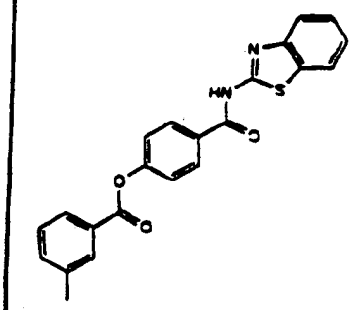
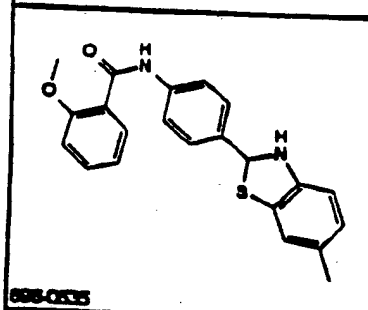
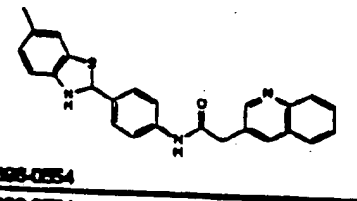
605-0662			
605-0662			
	165.876	uM	54.72
	16.588		159.21
301.43	3.318		113.97
	0.664		41.98
	0.133		38.28



605-0663			
605-0663			
	113.552	uM	-20.67
	11.355		201.56
440.326	2.271		12.55
	0.454		0.62
	0.091		-0.69



605-0606			
605-0606			
	178.349	uM	-28.16
	17.835		0.62
280.349	3.567		182.84
	0.713		118.95
	0.143		42.75

	464.979	2.151	188.84
	—	0.430	108.12
	—	0.096	37.18
			
606-0320			
606-0320		128.716 uM	
		12.872	-16.80
	388.445	2.574	87.23
		0.515	210.25
		0.103	73.35
			28.25
			
606-0535			
606-0535		132.810 uM	
		13.281	-10.41
	376.478	2.656	73.84
		0.531	199.80
		0.106	102.12
			35.72
			
606-0554			
606-0554		121.499 uM	
		12.150	-16.32
	411.527	2.430	105.48
		0.486	118.43
		0.097	53.88
			27.03

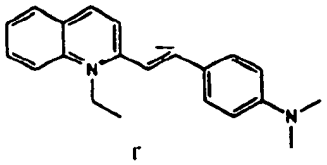
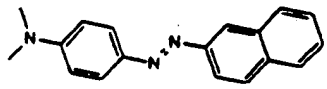
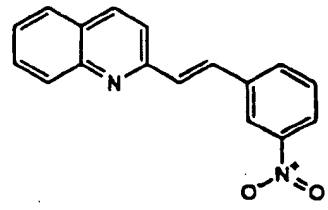
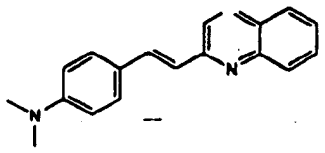
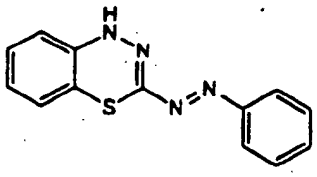
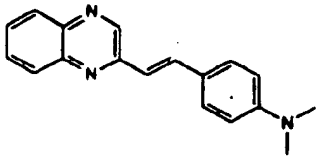
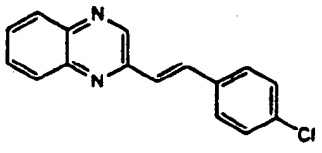
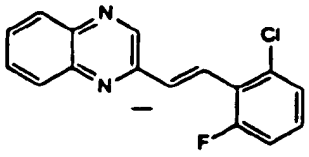
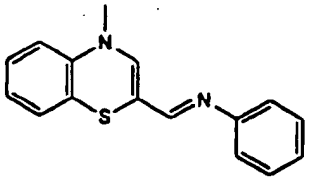
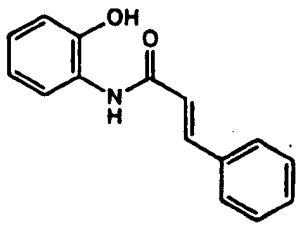
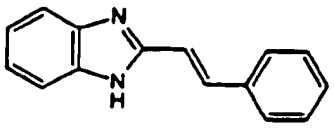
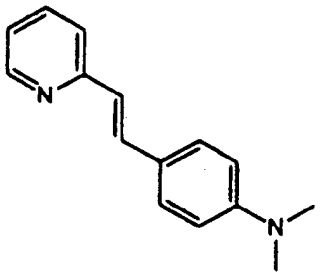
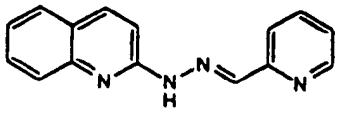
NNC#	MOL.WEIGHT	Concentration		% Response
				
50-0194	430.33			
50-0194		100.00	uM	-19.190
		31.25	uM	32.450
		9.77	uM	-14.240
		3.05	uM	-11.330
		953.67	nM	-12.790
		298.02	nM	-13.450
		93.13	nM	-12.290
		29.10	nM	-9.440
		9.09	nM	-6.450
		2.84	nM	-8.130
		888.18	pM	-3.320
				
50-0195	275.36			
50-0195		100.00	uM	-4.630
		31.25	uM	16.790
		9.77	uM	62.830
		3.05	uM	102.720
		953.67	nM	60.860
		298.02	nM	32.450
		93.13	nM	19.340
		29.10	nM	17.220
		9.09	nM	5.640
		2.84	nM	4.840
		888.18	pM	5.640
				
50-0196	276.30			
50-0196		100.00	uM	-16.210
		31.25	uM	-8.560
		9.77	uM	11.620
		3.05	uM	27.790
		953.67	nM	18.390
		298.02	nM	6.230
		93.13	nM	12.420
		29.10	nM	12.630
		9.09	nM	6.590
		2.84	nM	7.970
		888.18	pM	5.060

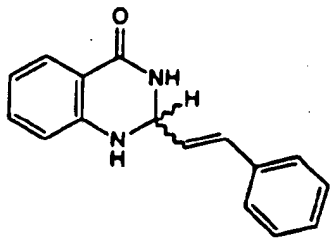
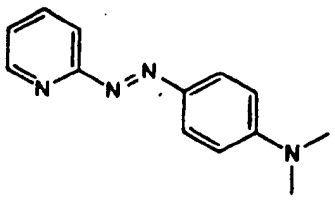
Figure 2

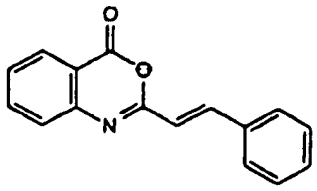
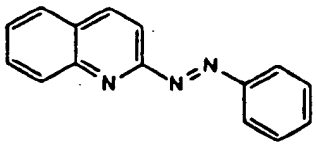
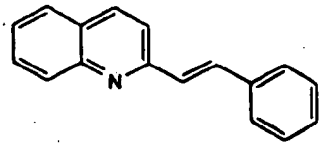
				
50-0197	274.37			
50-0197		100.00	uM	-18.250
		31.25	uM	-14.980
		9.77	uM	4.040
		3.05	uM	93.790
		953.67	nM	205.530
		298.02	nM	242.920
		93.13	nM	195.890
		29.10	nM	115.320
		9.09	nM	85.630
		2.84	nM	54.380
		888.18	pM	33.160
				
59-0008	254.32			
				
59-0019	59-0019			
59-0019		100.00	uM	-22.240
		31.25	uM	-22.670
		9.77	uM	-17.470
		3.05	uM	74.490
		953.67	nM	198.060
		298.02	nM	258.340
		93.13	nM	225.350
		29.10	nM	75.220
		9.09	nM	24.030
		2.84	nM	34.480
		888.18	pM	-3.740
				
59-0020	266.73			
59-0020		100.00	uM	-16.510
		31.25	uM	-16.040
		9.77	uM	-0.270
		3.05	uM	96.490
		953.67	nM	153.320
		298.02	nM	110.240
		93.13	nM	60.030

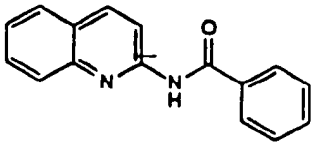
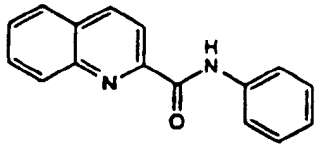
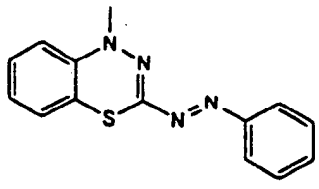
		29.10 nM	37.870
		9.09 nM	24.820
		2.84 nM	20.500
		888.18 pM	13.310

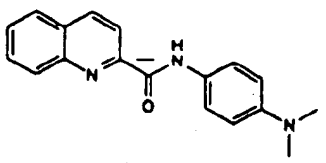
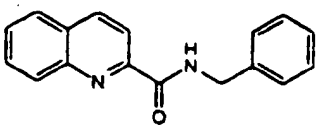
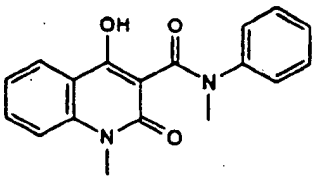
			
59-0021	284.72		
59-0021		100.00 uM	-16.310
		31.25 uM	-12.850
		9.77 uM	84.130
		3.05 uM	89.940
		953.67 nM	65.750
		298.02 nM	33.940
		93.13 nM	22.860
		29.10 nM	25.020
		9.09 nM	13.910
		2.84 nM	33.270
		888.18 pM	15.500
			
59-0022	266.37		
59-0022		100.00 uM	7.250
		31.25 uM	-2.070
		9.77 uM	-0.270
		3.05 uM	4.390
		953.67 nM	3.060
		298.02 nM	-1.600
		93.13 nM	-0.200
		29.10 nM	-3.270
		9.09 nM	1.130
		2.84 nM	2.590
		888.18 pM	2.460
			
59-0023	239.26		
59-0023		100.00 uM	-12.720
		31.25 uM	33.140
		9.77 uM	56.500
		3.05 uM	29.550
		953.67 nM	25.360
		298.02 nM	15.700
		93.13 nM	7.380
		29.10 nM	9.710
		9.09 nM	1.000
		2.84 nM	4.520
		888.18 pM	-0.010

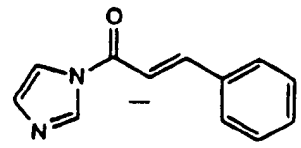
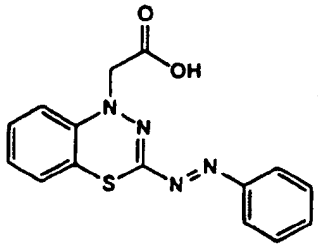
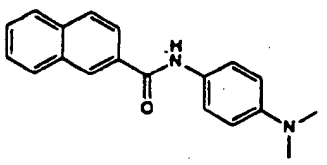
					
59-0024	220.28				
					
59-0025	224.31				
59-0025 .		100.00	uM	-25.590	
		31.25	uM	14.150	
		9.77	uM	50.690	
		3.05	uM	57.880	
		953.67	nM	38.900	
		298.02	nM	28.530	
		93.13	nM	19.660	
		29.10	nM	17.490	
		9.09	nM	-0.600	
		2.84	nM	-4.190	
		888.18	pM	4.670	
					
59-0026	248.29				
59-0026		100.00	uM	-29.830	
		31.25	uM	-9.440	
		9.77	uM	-10.470	
		3.05	uM	46.220	
		953.67	nM	107.760	
		298.02	nM	86.720	
		93.13	nM	36.850	
		29.10	nM	26.720	
		9.09	nM	8.520	
		2.84	nM	-1.240	
		888.18	pM	4.020	

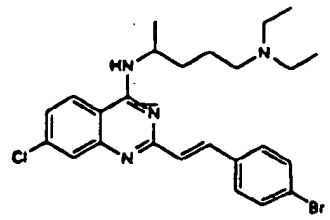
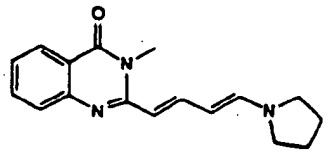
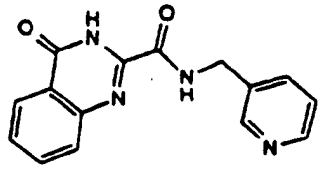
					
59-0027	250.30				
59-0027		100.00	uM	89.810	
		31.25	uM	54.670	
		9.77	uM	44.940	
		3.05	uM	23.780	
		953.67	nM	8.380	
		298.02	nM	6.330	
		93.13	nM	7.360	
		29.10	nM	3.380	
		9.09	nM	-1.620	
		2.84	nM	-3.670	
		888.18	pM	-0.720	
					
59-0028	226.28				
59-0028		100.00	uM	-26.750	
		31.25	uM	-16.740	
		9.77	uM	29.550	
		3.05	uM	100.580	
		953.67	nM	54.940	
		298.02	nM	31.340	
		93.13	nM	7.500	
		29.10	nM	7.500	
		9.09	nM	7.880	
		2.84	nM	3.140	
		888.18	pM	4.670	

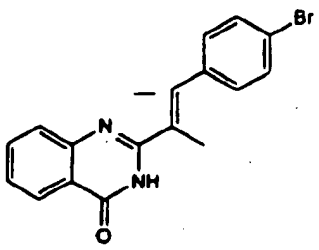
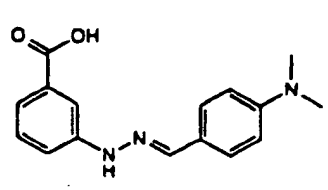
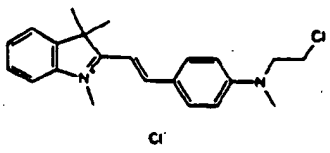
			
59-0029	249.27		
59-0029		100.00 uM	-15.160
		31.25 uM	41.940
		9.77 uM	36.630
		3.05 uM	7.120
		953.67 nM	21.880
		298.02 nM	15.540
		93.13 nM	1.810
		29.10 nM	1.370
		9.09 nM	12.140
		2.84 nM	-4.230
		888.18 pM	9.040
			
59-0030	233.28		
59-0030		100.00 uM	-27.970
		31.25 uM	-22.830
		9.77 uM	-5.420
		3.05 uM	57.280
		953.67 nM	72.620
		298.02 nM	53.000
		93.13 nM	29.990
		29.10 nM	14.630
		9.09 nM	3.870
		2.84 nM	6.970
		888.18 pM	1.810
			
59-0031	231.30		
59-0031		100.00 uM	-25.790
		31.25 uM	-17.610
		9.77 uM	20.840
		3.05 uM	87.380
		953.67 nM	49.320
		298.02 nM	43.110
		93.13 nM	29.530
		29.10 nM	1.810
		9.09 nM	1.220
		2.84 nM	-0.550
		888.18 pM	4.160

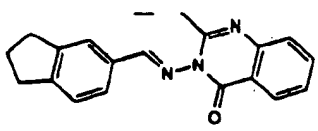
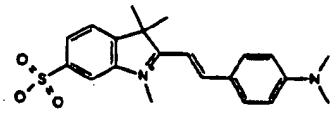
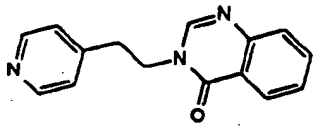
			
59-0032	248.29		
59-0032		100.00 uM	-7.780
		31.25 uM	40.750
		9.77 uM	42.620
		3.05 uM	25.700
		953.67 nM	31.170
		298.02 nM	34.410
		93.13 nM	3.570
		29.10 nM	4.320
		9.09 nM	-10.000
		2.84 nM	5.650
		888.16 pM	11.990
			
59-0033	248.29		
59-0033		100.00 uM	-28.160
		31.25 uM	-11.590
		9.77 uM	55.300
		3.05 uM	49.710
		953.67 nM	47.410
		298.02 nM	0.250
		93.13 nM	7.980
		29.10 nM	-8.940
		9.09 nM	-7.630
		2.84 nM	-0.400
		888.16 pM	-5.980
			
59-0034	268.34		
59-0034		100.00 uM	-28.51
		31.25 uM	24
		9.77 uM	73.58
		3.05 uM	37.91
		953.67 nM	20.09
		298.02 nM	16.87
		93.13 nM	15.23
		29.10 nM	28.83
		9.09 nM	9.08
		2.84 nM	23.02
		888.16 pM	-0.32

			
59-0035	291.36		
59-0035		100.00uM	-14.92
		31.25uM	29.17
		9.77uM	15.67
		3.05uM	18.8
		953.67nM	3.88
		298.02nM	6.15
		93.13nM	3.22
		29.10nM	-10.03
		9.09nM	15.58
		2.84nM	-3.56
		888.18pM	-7.13
			
59-0036	262.31		
59-0036		100.00uM	-0.98
		31.25uM	-3.25
		9.77uM	-4.54
		3.05uM	-1.95
		953.67nM	0.32
		298.02nM	-6.49
		93.13nM	-17.19
		29.10nM	-0.66
		9.09nM	-5.52
		2.84nM	-9.4
		888.18pM	-16.53
			
59-0037	308.00		
59-0037		100.00uM	-10.69
		31.25uM	-11.99
		9.77uM	-10.03
		3.05uM	-19.11
		953.67nM	-9.4
		298.02nM	2.27
		93.13nM	-2.9
		29.10nM	-10.69
		9.09nM	2.59
		2.84nM	0.66
		888.18pM	-2.59

			
59-0038	291.36		
59-0038		100.00 uM	-23.430
		31.25 uM	-8.390
		9.77 uM	-0.100
		3.05 uM	-2.860
		953.67 nM	-2.240
		298.02 nM	3.900
		93.13 nM	6.350
		29.10 nM	1.150
		9.09 nM	6.960
		2.84 nM	-4.390
		888.18 pM	-0.380
			
59-0039	312.35		
59-0039		100.00 uM	14.170
		31.25 uM	7.620
		9.77 uM	1.940
		3.05 uM	-3.140
		953.67 nM	-7.770
		298.02 nM	-5.980
		93.13 nM	-8.620
		29.10 nM	-2.390
		9.09 nM	-16.580
		2.84 nM	-4.480
		888.18 pM	-0.450
			
59-0040	290.37		
59-0040		100.00 uM	-20.400
		31.25 uM	-17.310
		9.77 uM	-8.110
		3.05 uM	32.180
		953.67 nM	36.180
		298.02 nM	17.440
		93.13 nM	2.040
		29.10 nM	10.350
		9.09 nM	-6.070
		2.84 nM	6.960
		888.18 pM	13.440

			
59-0041	501.90		
59-0041		100.00 uM	-18.37
		31.25 uM	-17.33
		9.77 uM	-5.11
		3.05 uM	3.31
		953.67 nM	-0.77
		298.02 nM	-1.56
		93.13 nM	3.55
		29.10 nM	-11.24
		9.09 nM	0.25
		2.84 nM	-0.27
		888.18 pM	2.02
			
59-0042	281.36		
59-0042		100.00 uM	163.51
		31.25 uM	-7.67
		9.77 uM	9.41
		3.05 uM	0.75
		953.67 nM	6.11
		298.02 nM	3.82
		93.13 nM	2.54
		29.10 nM	4.07
		9.09 nM	-9.73
		2.84 nM	-0.02
		888.18 pM	18.37
			
59-0043	280.29		
59-0043		100.00 uM	20.66
		31.25 uM	7.41
		9.77 uM	-1.29
		3.05 uM	-2.31
		953.67 nM	1.54
		298.02 nM	-0.79
		93.13 nM	1.52
		29.10 nM	2.79
		9.09 nM	-0.27
		2.84 nM	8.92
		888.18 pM	-4.34

				
59-0044	341.21			
59-0044		100.00 uM	7.38	
		31.25 uM	11.72	
		9.77 uM	12.49	
		3.05 uM	-0.52	
		953.67 nM	0.5	
		298.02 nM	6.11	
		93.13 nM	-1.54	
		29.10 nM	19.14	
		9.09 nM	7.13	
		2.84 nM	-2.06	
		888.18 pM	5.84	
				
59-0045	283.33			
59-0045		100.00 uM	52.37	64.460
		31.25 uM	148.43	192.960
		9.77 uM	204.47	422.540
		3.05 uM	280.3	437.020
		953.67 nM	254.62	410.890
		298.02 nM	218.21	266.090
		93.13 nM	196.98	183.730
		29.10 nM	96.06	80.440
		9.09 nM	67.35	55.530
		2.84 nM	52.99	44 160
				
59-0046	389.37			
59-0046		100.00 uM	79.33	
		31.25 uM	2.24	
		9.77 uM	-1.67	
		3.05 uM	-6.18	
		953.67 nM	0.001	
		298.02 nM	-3.63	
		93.13 nM	-0.84	
		29.10 nM	-8.42	
		9.09 nM	3.92	
		2.84 nM	0.3	
		888.18 pM	5.61	

					
59-0047	303.37				
59-0047		100.00	uM	-6.73	
		31.25	uM	10.38	
		9.77	uM	-6.16	
		3.05	uM	-1.39	
		953.67	nM	-10.11	
		298.02	nM	-4.49	
		93.13	nM	-7.28	
		29.10	nM	-12.34	
		9.09	nM	-3.08	
		2.84	nM	-2.26	
		888.18	pM	-5.34	
					
59-0048	384.50				
59-0048		100.00	uM	-6.73	
		31.25	uM	0.27	
		9.77	uM	-5.61	
		3.05	uM	-2.26	
		953.67	nM	-12.89	
		298.02	nM	-1.69	
		93.13	nM	-4.77	
		29.10	nM	-8.14	
		9.09	nM	-3.92	
		2.84	nM	-11.2	
		888.18	pM	-4.77	
					
59-0049	251.29				
59-0049		100.00	uM	4.49	
		31.25	uM	0	
		9.77	uM	-4.77	
		3.05	uM	1.96	
		953.67	nM	8.69	
		298.02	nM	-5.04	
		93.13	nM	-2.24	
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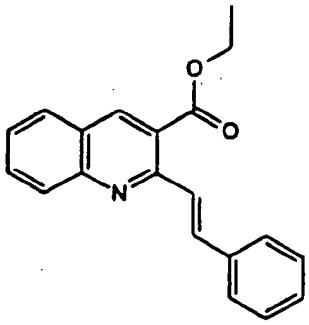
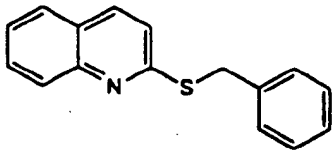
		9.09	nM	-4.49	
		2.84	nM	2.24	
		888.18	pM	-0.3	

					
59-0050	303.36				
59-0050		100.00	uM	45.79	
		31.25	uM	10.02	
		9.77	uM	11.29	
		3.05	uM	-4.68	
		953.67	nM	-6.92	
		298.02	nM	-5.65	
		93.13	nM	1.69	
		29.10	nM	-7.57	
		9.09	nM	-12.05	
		2.84	nM	-13.63	
		888.18	pM	5.2	
					
59-0051	251.35				
59-0051		100.00	uM	32.36	
		31.25	uM	-18.42	
		9.77	uM	-0.55	
		3.05	uM	-13.94	
		953.67	nM	-12.02	
		298.02	nM	-14.59	
		93.13	nM	-7.55	
		29.10	nM	-11.4	
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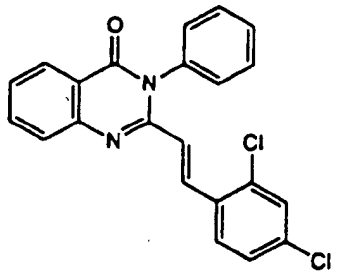
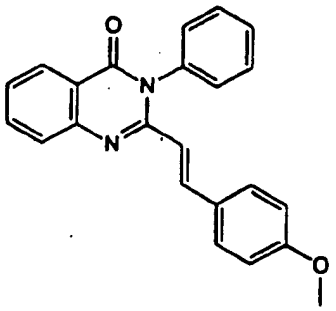
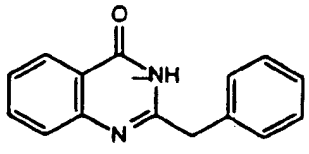
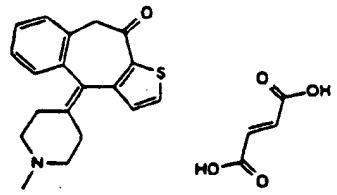
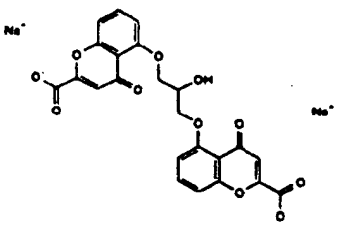
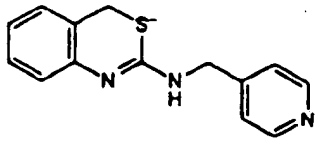
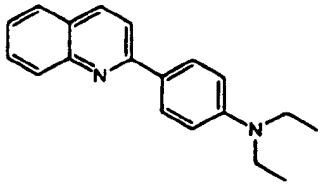
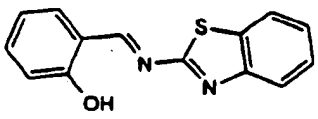
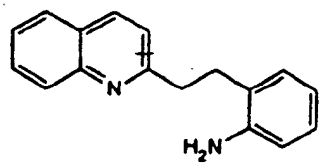
		2.84	nM	-10.74	
		888.18	pM	-20.03	

					
59-0052	393.28				
59-0052		100.00	uM	-21.62	
		31.25	uM	-13.32	
		9.77	uM	-21.31	
		3.05	uM	-11.08	
		953.67	nM	-20.66	
		298.02	nM	-17.14	
		93.13	nM	-16.49	
		29.10	nM	-11.4	
		9.09	nM	-10.74	
		2.84	nM	-11.08	
		888.18	pM	-14.59	
					
59-0053	354.41				
59-0053		100.00	uM	-17.14	
		31.25	uM	-21.31	
		9.77	uM	-9.47	
		3.05	uM	-11.08	
		953.67	nM	-0.83	
		298.02	nM	-11.4	
		93.13	nM	-9.47	
		29.10	nM	-19.72	
		9.09	nM	-18.45	
		2.84	nM	-10.09	
		888.18	pM	-2.76	

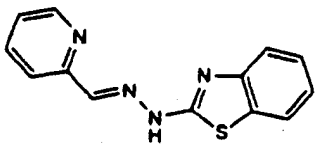
			
59-0054	236.28		
59-0054		100.00 uM	-20.04
		31.25 uM	-6.95
		9.77 uM	8.3
		3.05 uM	-3.37
		953.67 nM	-2.4
		298.02 nM	-0.99
		93.13 nM	-0.99
		29.10 nM	-1.94
		9.09 nM	5.92
		2.84 nM	-2.17
		888.18 pM	-9.31
			
59-0055	425.51		
59-0055		100.00 uM	-13.76
		31.25 uM	-9.51
		9.77 uM	-2.02
		3.05 uM	3.24
		953.67 nM	-6.27
		298.02 nM	-4.05
		93.13 nM	-1.62
		29.10 nM	-7.49
		9.09 nM	-7.09
		2.84 nM	-3.04
			
59-0056	512.34		
59-0056		100.00 uM	-1.42
		31.25 uM	-4.87
		9.77 uM	0.18
		3.05 uM	3.84
		953.67 nM	-5.07
		298.02 nM	-7.29
		93.13 nM	0.001
		29.10 nM	-4.25
		9.09 nM	-1.02
		2.84 nM	-3.85

		9.09 nM	8.070
		2.84 nM	0.440
 59-0063			
59-0063		100.00 uM	-2.510
		31.25 uM	-6.130
		9.77 uM	-8.950
		3.05 uM	-8.020
		953.67 nM	-8.010
		298.02 nM	-2.520
		93.13 nM	-5.810
		29.10 nM	-3.450
		9.09 nM	-4.390
		2.84 nM	-6.280
 59-0064			
59-0064		100.00 uM	-23.090
		31.25 uM	-21.040
		9.77 uM	78.400
		3.05 uM	155.220
		953.67 nM	113.120
		298.02 nM	30.640
		93.13 nM	15.240
		29.10 nM	22.150
		9.09 nM	-0.770
		2.84 nM	4.410
 59-0065			
59-0065		100.00 uM	-2.030
		31.25 uM	-2.980
		9.77 uM	-15.240
		3.05 uM	-15.400
		953.67 nM	-15.240
		298.02 nM	-10.520
		93.13 nM	-13.830
		29.10 nM	-5.810
		9.09 nM	-3.620
		2.84 nM	-7.070



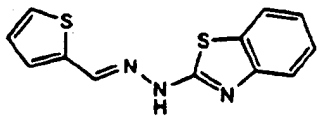
59-0066

59-0066		100.00	uM	10.060	
		31.25	uM	2.680	
		9.77	uM	10.850	
		3.05	uM	14.610	
		953.67	nM	0.950	
		298.02	nM	3.780	
		93.13	nM	1.730	
		29.10	nM	-2.820	
		9.09	nM	-2.820	
		2.84	nM	-3.920	



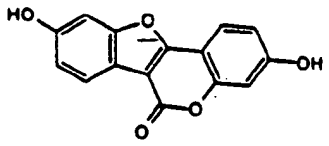
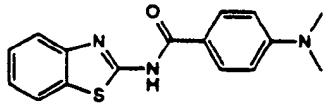
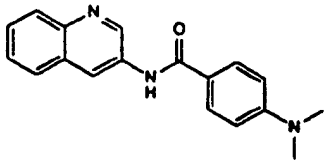
59-0067

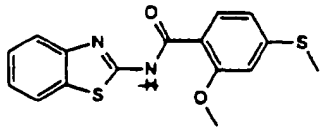
59-0067		100.00	uM	-24.040	
		31.25	uM	-24.890	
		9.77	uM	-1.450	
		3.05	uM	60.900	
		953.67	nM	133.860	
		298.02	nM	75.330	
		93.13	nM	28.760	
		29.10	nM	20.070	
		9.09	nM	4.980	
		2.84	nM	4.450	



59-0068

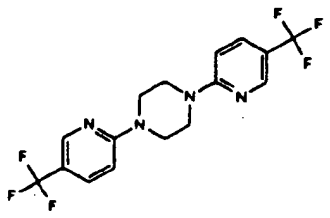
59-0068		100.00	uM	-22.130	
		31.25	uM	-7.880	
		9.77	uM	93.900	
		3.05	uM	81.060	
		953.67	nM	22.330	
		298.02	nM	17.300	
		93.13	nM	8.460	
		29.10	nM	-3.530	
		9.09	nM	-4.230	
		2.84	nM	-6.140	

 59-0069			
59-0069	100.00	uM	5.490
	31.25	uM	9.670
	9.77	uM	16.090
	3.05	uM	-7.180
	953.67	nM	-2.840
	298.02	nM	-3.710
	93.13	nM	-11.180
	29.10	nM	-5.790
	9.09	nM	-7.180
	2.84	nM	-4.750
 59-0070			
59-0070	100.00	uM	-25.930
	31.25	uM	-23.000
	9.77	uM	36.060
	3.05	uM	214.280
	953.67	nM	158.530
	298.02	nM	72.890
	93.13	nM	20.940
	29.10	nM	7.760
	9.09	nM	7.590
	2.84	nM	-8.400
 59-0071			
59-0071	100.00	uM	-18.650
	31.25	uM	-15.540
	9.77	uM	17.060
	3.05	uM	176.090
	953.67	nM	76.070
	298.02	nM	31.260
	93.13	nM	16.410
	29.10	nM	4.870
	9.09	nM	-7.330
	2.84	nM	-4.660



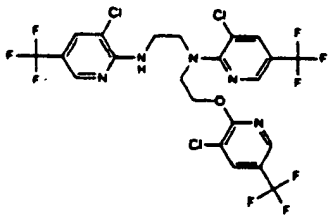
59-0072

59-0072	100.00	uM	-19.750
	31.25	uM	-18.650
	9.77	uM	-18.430
	3.05	uM	-15.770
	953.67	nM	9.970
	298.02	nM	74.740
	93.13	nM	175.430
	29.10	nM	213.580
	9.09	nM	164.320
	2.84	nM	119.100
	888.16	pM	60.770



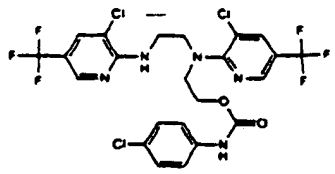
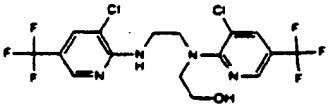
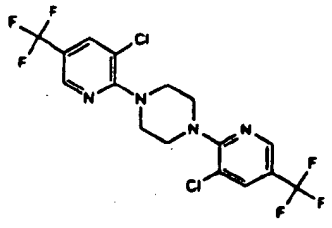
59-0073

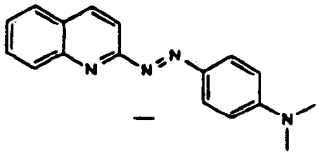
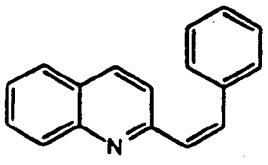
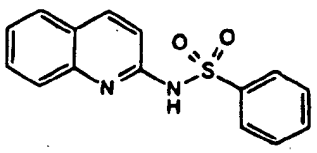
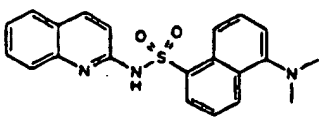
59-0073	100.00	uM	-3.010
	31.25	uM	-4.830
	9.77	uM	-9.660
	3.05	uM	-4.680
	953.67	nM	-6.500
	298.02	nM	-2.510
	93.13	nM	7.140
	29.10	nM	0.97
	9.09	nM	-5.5
	2.84	nM	5.3

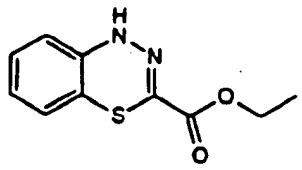
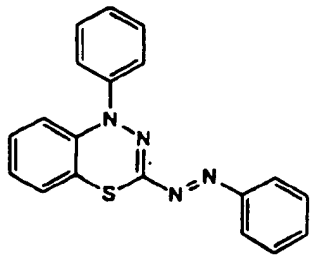
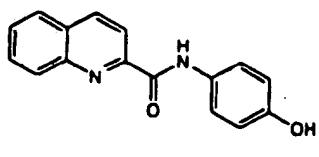


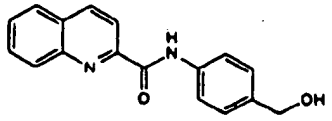
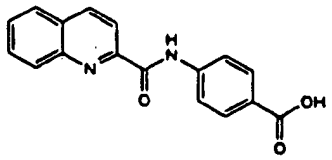
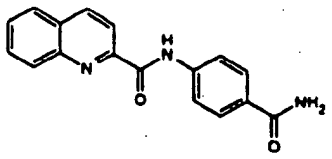
59-0074

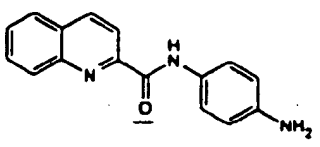
59-0074	100.00	uM	-2.85
	31.25	uM	2.14
	9.77	uM	-4.85
	3.05	uM	-3.5
	953.67	nM	-4.85
	298.02	nM	9.95
	93.13	nM	4.47
	29.10	nM	-8
	9.09	nM	-4.17
	2.84	nM	6.97

 59-0075					
59-0075		100.00	uM	-0.68	
		31.25	uM	-10.16	
		9.77	uM	-5.35	
		3.05	uM	-6.5	
		953.67	nM	-0.85	
		298.02	nM	5.97	
		93.13	nM	0.97	
		29.10	nM	-2.35	
		9.09	nM	0.32	
		2.84	nM	10.47	
 59-0076					
59-0076		100.00	uM	-19.12	
		31.25	uM	9.29	
		9.77	uM	10.63	
		3.05	uM	22.43	
		953.67	nM	19.93	
		298.02	nM	3.47	
		93.13	nM	19.93	
		29.10	nM	10.63	
		9.09	nM	14.28	
		2.84	nM	11.3	
 59-0077					
59-0077		100.00	uM	-20.96	
		31.25	uM	-16.23	
		9.77	uM	-10.58	
		3.05	uM	-11.96	
		953.67	nM	-19.44	
		298.02	nM	-17.3	
		93.13	nM	-13.79	
		29.10	nM	-15.62	
		9.09	nM	-14.09	
		2.84	nM	-14.4	

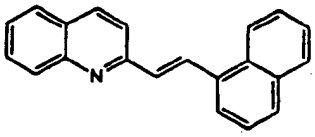
			
59-0078			
		100.00 uM	-26.540
		31.25 uM	-22.560
		9.77 uM	71.530
		3.05 uM	207.960
		953.67 nM	379.230
		298.02 nM	241.460
		93.13 nM	136.100
		29.10 nM	84.020
		9.09 nM	50.350
		2.84 nM	56.600
		0.80 nM	92.520
			
59-0079			
59-0079		100.00 uM	-34.980
		31.25 uM	-21.390
		9.77 uM	37.200
		3.05 uM	122.580
		953.67 nM	69.010
		298.02 nM	64.000
		93.13 nM	46.490
		29.10 nM	30.310
		9.09 nM	33.490
		2.84 nM	29.760
			
59-0080			
59-0080		100.00 uM	5.390
		31.25 uM	5.560
		9.77 uM	6.440
		3.05 uM	2.440
		953.67 nM	-5.030
		298.02 nM	7.660
		93.13 nM	-3.630
		29.10 nM	3.650
		9.09 nM	1.050
		2.84 nM	6.940
			—
59-0081			

59-0081		100.00 uM	62.840
		31.25 uM	11.300
		9.77 uM	-8.670
		3.05 uM	2.440
		953.67 nM	-5.200
		298.02 nM	-2.080
		93.13 nM	1.220
		29.10 nM	-2.250
		9.09 nM	1.050
		2.84 nM	-3.300
 59-0082			
59-0082		100.00 uM	111.79
		31.25 uM	62.68
		9.77 uM	32.36
		3.05 uM	9.11
		953.67 nM	-10.62
		298.02 nM	-1.86
		93.13 nM	-6.89
		29.10 nM	-3.91
		9.09 nM	2.22
		2.84 nM	16.36
 59-0083			
59-0083		100.00 uM	48.93
		31.25 uM	40.91
		9.77 uM	25.85
		3.05 uM	17.85
		953.67 nM	8.55
		298.02 nM	3.9
		93.13 nM	2.05
		29.10 nM	7.99
		9.09 nM	-3.91
		2.84 nM	3.35
 59-0084			
59-0084		100.00 uM	37.670
		31.25 uM	26.050
		9.77 uM	9.210
		3.05 uM	10.070

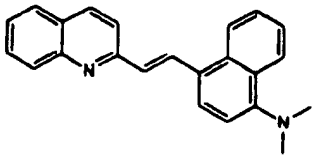
		953.67	nM	21.700	
		298.02	nM	5.900	
		93.13	nM	4.870	
		29.10	nM	-10.920	
		9.09	nM	10.080	
		2.84	nM	-2.080	
 59-0085					
59-0085		100.00	uM	17.070	
		31.25	uM	41.890	
		9.77	uM	18.500	
		3.05	uM	20.340	
		953.67	nM	22.490	
		298.02	nM	8.090	
		93.13	nM	11.790	
		29.10	nM	1.240	
		9.09	nM	-0.760	
		2.84	nM	5.940	
 59-0086					
59-0086		100.00	uM	30.750	
		31.25	uM	31.190	
		9.77	uM	14.790	
		3.05	uM	13.500	
		953.67	nM	14.080	
		298.02	nM	3.940	
		93.13	nM	9.370	
		29.10	nM	-2.610	
		9.09	nM	-5.040	
		2.84	nM	1.530	
 59-0087					
59-0087		100.00	uM	10.660	
		31.25	uM	11.080	
		9.77	uM	3.100	
		3.05	uM	-1.320	
		953.67	nM	17.070	
		298.02	nM	7.950	
		93.13	nM	-4.460	
		29.10	nM	4.510	
		9.09	nM	-0.470	
		2.84	nM	9.660	



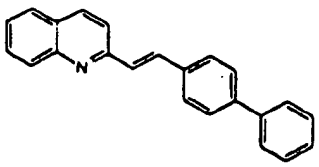
59-0088			
59-0088	100.00	uM	
	31.25	uM	
	9.77	uM	
	3.05	uM	
	953.67	nM	
	298.02	nM	
	93.13	nM	
	29.10	nM	
	9.09	nM	
	2.84	nM	



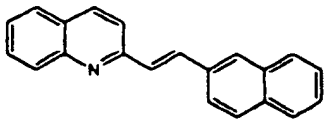
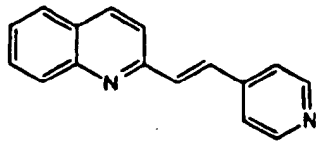
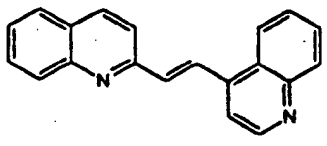
59-0089			
59-0089	100.00	uM	60.09
	31.25	uM	116.25
	9.77	uM	65.84
	3.05	uM	36.1
	953.67	nM	37.96
	298.02	nM	18.42
	93.13	nM	6.33
	29.10	nM	13.58
	9.09	nM	0.75
	2.84	nM	-5.77

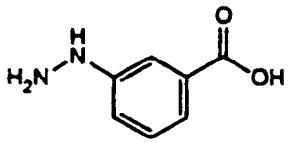
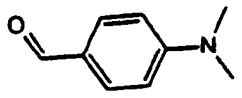
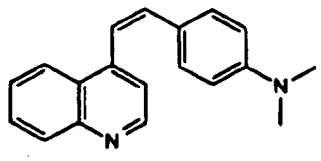


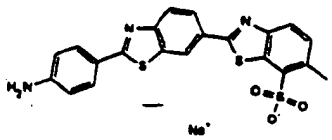
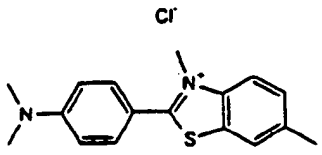
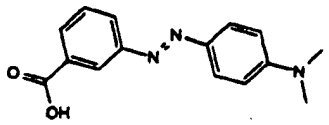
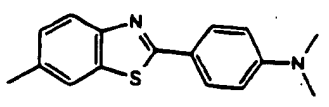
59-0090			
59-0090	100.00	uM	32.77
	31.25	uM	24.63
	9.77	uM	19.5
	3.05	uM	41.31
	953.67	nM	9.8
	298.02	nM	-1.76
	93.13	nM	3.53
	29.10	nM	2.95
	9.09	nM	2.95
	2.84	nM	7.8

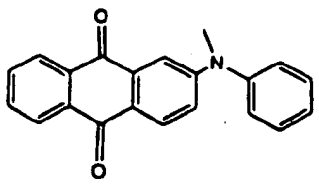
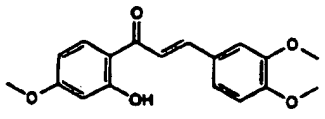
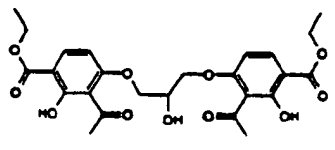


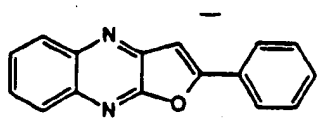
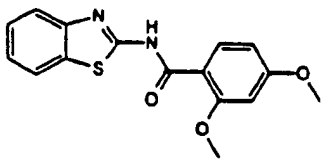
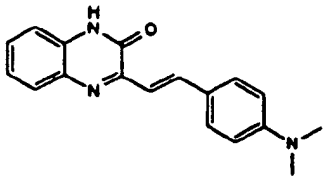
59-0091			
59-0091	100.00	uM	0.26
	31.25	uM	13.54

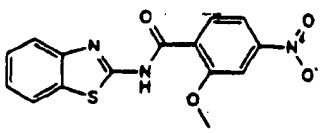
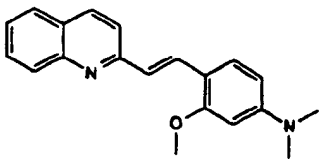
		9.77 uM	95.94
		3.05 uM	87.71
		953.67 nM	44.17
		298.02 nM	38.26
		93.13 nM	23.87
		29.10 nM	21.65
		9.09 nM	10.95
		2.84 nM	20.92
 59-0092			
59-0092		100.00 uM	-11.56
		31.25 uM	17.84
		9.77 uM	50.19
		3.05 uM	25.84
		953.67 nM	14.4
		298.02 nM	6.77
		93.13 nM	8.62
		29.10 nM	2.22
		9.09 nM	8.38
		2.84 nM	1
 59-0093			
59-0093		100.00 uM	-11.67
		31.25 uM	15.02
		9.77 uM	35.44
		3.05 uM	29.89
		953.67 nM	22.88
		298.02 nM	19.56
		93.13 nM	5.18
		29.10 nM	7.39
		9.09 nM	4.56
		2.84 nM	5.9
 59-0094			
59-0094		100.00 uM	-17.69
		31.25 uM	45.15
		9.77 uM	24.97
		3.05 uM	19.81
		953.67 nM	9.35
		298.02 nM	1.36
		93.13 nM	9.24
		29.10 nM	-0.48
		9.09 nM	6.16
		2.84 nM	1.61

		93.13 nM	10.68
		29.10 nM	5.89
		9.09 nM	5.45
—		2.84 nM	10.24
		0.80 nM	4.14
 59-0111	152.15		
		100.00 uM	23.360
		31.25 uM	22.330
		9.77 uM	12.260
		3.05 uM	5.390
		953.67 nM	2.190
		298.02 nM	1.230
		93.13 nM	2.430
		29.10 nM	6.350
		9.09 nM	4.350
		2.84 nM	4.350
		0.80 nM	3.230
 59-0112	149.19		
		100.00 uM	2.670
		31.25 uM	4.670
		9.77 uM	2.750
		3.05 uM	3.790
		953.67 nM	4.270
		298.02 nM	1.150
		93.13 nM	9.630
		29.10 nM	0.920
		9.09 nM	0.510
		2.84 nM	12.900
		0.80 nM	2.990
 59-0113	274.37		
		100.00 uM	22.010
		31.25 uM	25.940
		9.77 uM	7.500
		3.05 uM	3.070
		953.67 nM	-0.760
		298.02 nM	-4.690
		93.13 nM	-4.780
		29.10 nM	5.090
		9.09 nM	0.150
		2.84 nM	-0.250
		0.80 nM	0.150

					
59-0114	475.54				
		100.00	uM	52.030	
		31.25	uM	36.120	
		9.77	uM	25.840	
		3.05	uM	16.670	
		953.67	nM	12.540	
		298.02	nM	9.420	
		93.13	nM	-1.060	
		29.10	nM	2.160	
		9.09	nM	-6.000	
		2.84	nM	2.470	
		0.80	nM	-1.460	
					
59-0115	318.87				
		100.00	uM	73.700	
		31.25	uM	2.770	
		9.77	uM	-10.430	
		3.05	uM	-12.340	
		953.67	nM	-13.750	
		298.02	nM	-13.960	
		93.13	nM	-11.940	
		29.10	nM	-9.830	
		9.09	nM	-8.820	
		2.84	nM	-0.950	
		0.80	nM	-0.050	
					
59-0116	269.30				
		100.00	uM	31.380	
		31.25	uM	109.060	
		9.77	uM	231.070	
		3.05	uM	240.670	
		953.67	nM	132.020	
		298.02	nM	75.820	
		93.13	nM	53.250	
		29.10	nM	47.500	
		9.09	nM	39.440	
		2.84	nM	42.170	
		0.80	nM	31.160	
				—	
59-0117	268.38				
		100.00	uM	-68.520	

		31.25 uM	-7.450
		9.77 uM	111.630
		3.05 uM	64.340
		953.67 nM	4.740
		298.02 nM	-19.270
		93.13 nM	-26.660
		29.10 nM	-28.880
		9.09 nM	-42.180
		2.84 nM	-41.300
		0.80 nM	-39.220
 59-0118	313.36		
		100.00 uM	-67.170
		31.25 uM	-56.580
		9.77 uM	-58.060
		3.05 uM	-55.720
		953.67 nM	-48.200
		298.02 nM	-50.300
		93.13 nM	-33.310
		29.10 nM	-47.340
		9.09 nM	-49.310
		2.84 nM	-56.200
		0.80 nM	-57.310
 59-0119	314.34		
		100.00 uM	167.500
		31.25 uM	-29.240
		9.77 uM	-57.800
		3.05 uM	-52.030
		953.67 nM	-54.240
		298.02 nM	-53.870
		93.13 nM	-38.110
		29.10 nM	-55.100
		9.09 nM	-52.270
		2.84 nM	-53.500
		0.80 nM	-43.650
 59-0120	504.49		
		100.00 uM	-82.790
		31.25 uM	-80.470
		9.77 uM	-66.800
		3.05 uM	-60.790
		953.67 nM	-54.240
		298.02 nM	-45.250
		93.13 nM	-50.660

		2.84	nM	6.27		
		0.80	nM	3.55		
 59-0146	246.27					
		100.00	uM	-63.05		
		31.25	uM	4.42		
		9.77	uM	-13.73		
		3.05	uM	-16.45		
		953.67	nM	-35.47		
		298.02	nM	-51.25		
		93.13	nM	-50.13		
		29.10	nM	-42.92		
		9.09	nM	-45.64		
		2.84	nM	-56.58		
		0.80	nM	-39.68		
 59-0147	314.36					
		100.00	uM	-85		
		31.25	uM	-85		
		9.77	uM	-80.29		
		3.05	uM	-41.67		
		953.67	nM	78.69		
		298.02	nM	269.13		
		93.13	nM	323.59		
		29.10	nM	339.88		
		9.09	nM	270.48		
		2.84	nM	245.58		
		0.80	nM	180.33		
 59-0148	291.35					
		100.00	uM	-68.36		
		31.25	uM	-36.33		
		9.77	uM	-2.3		
		3.05	uM	12.12		
		953.67	nM	-2.42		
		298.02	nM	-16.21		
		93.13	nM	-30.87		
		29.10	nM	-35.56		
		9.09	nM	-39.07		
		2.84	nM	-41.18		
		0.80	nM	-45.53		

						
59-0149	329.33					
		100.00	uM	-16.9		
		31.25	uM	-1.8		
		9.77	uM	-0.53		
		3.05	uM	15.29		
		953.67	nM	78.78		
		298.02	nM	163.5		
		93.13	nM	223.57		
		29.10	nM	173.93		
		9.09	nM	122.3		
		2.84	nM	98.02		
		0.80	nM	69.06		
						
59-0150	304.39					
		100.00	uM	63.32		
		31.25	uM	193.53		
		9.77	uM	419.26		
		3.05	uM	497.21		
		953.67	nM	295.19		
		298.02	nM	193.35		
		93.13	nM	99.46		
		29.10	nM	69.96		
		9.09	nM	59		
		2.84	nM	52.16		
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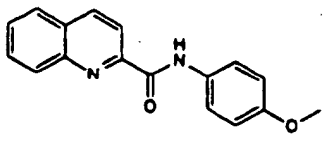
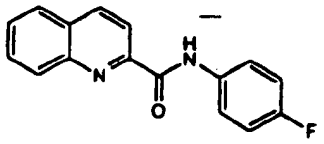
						
59-0151	278.311					
59-0151		100.00	uM	-6.660		
		31.25	uM	16.240		
		9.77	uM	18.300		
		3.05	uM	11.690		
		953.67	nM	8.500		
		298.02	nM	9.070		
		93.13	nM	6.110		
		29.10	nM	5.860		
		9.09	nM	7.700		
		2.84	nM	2.000		
		0.80	nM	1.210		

						
59-0152	266.275					
59-0152		100.00	uM	-6.890		
		31.25	uM	12.490		
		9.77	uM	21.950		
		3.05	uM	12.820		
		953.67	nM	7.350		
		298.02	nM	4.290		
		93.13	nM	9.750		
		29.10	nM	4.860		
		9.09	nM	1.320		
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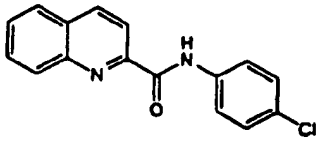
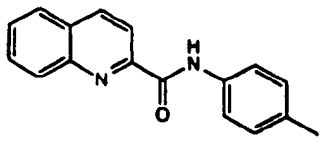
						
59-0153	282.73					
59-0153		100.00	uM	-4.150		
		31.25	uM	-0.390		
		9.77	uM	11.120		
		3.05	uM	14.540		
		953.67	nM	9.520		
		298.02	nM	11.570		
		93.13	nM	-0.160		
		29.10	nM	1.550		
		9.09	nM	-0.960		
		2.84	nM	4.730		
		0.80	nM	5.650		
						
59-0154	262.312					
59-0154		100.00	uM	0.290		
		31.25	uM	24.670		
		9.77	uM	15.680		
		3.05	uM	14.540		
		953.67	nM	13.170		
		298.02	nM	5.540		
		93.13	nM	2.690		
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		9.09	nM	2.460		
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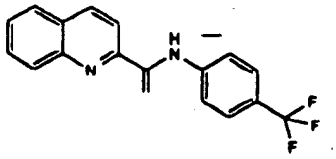
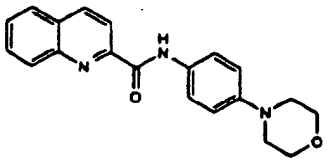
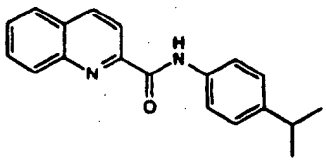
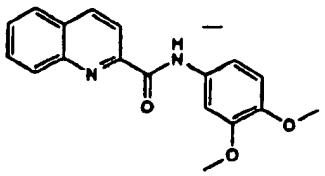
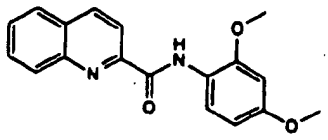
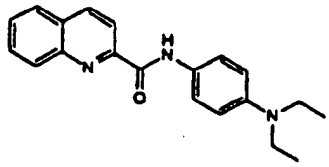
		0.80	nM	1.890		

						
59-0155	316.282					
59-0155		100.00	uM	-2.950		
		31.25	uM	1.900		
		9.77	uM	-0.450		
		3.05	uM	-0.220		
		953.67	nM	0.690		
		298.02	nM	5.090		
		93.13	nM	-3.250		
		29.10	nM	0.530		
		9.09	nM	-1.900		
		2.84	nM	9.480		
		0.80	nM	-1.130		
						
59-0156	333.391					
59-0156		100.00	uM	5.840		
		31.25	uM	2.050		
		9.77	uM	7.960		
		3.05	uM	6.890		
		953.67	nM	-0.370		
		298.02	nM	-1.880		
		93.13	nM	-3.550		
		29.10	nM	-7.340		
		9.09	nM	-1.590		
		2.84	nM	2.650		
		0.80	nM	2.500		
						
59-0157	290.366					
59-0157		100.00	uM	-6.440		
		31.25	uM	14.920		
		9.77	uM	19.930		
		3.05	uM	11.440		
		953.67	nM	8.570		
		298.02	nM	-7.190		
		93.13	nM	0.080		
		29.10	nM	-0.230		
		9.09	nM	-4.460		
		2.84	nM	2.200		
		0.80	nM	9.920		

						
59-0158	308.337					
59-0158		100.00	uM	-5.980		
		31.25	uM	3.720		
		9.77	uM	16.140		
		3.05	uM	27.060		
		953.67	nM	9.930		
		298.02	nM	11.900		
		93.13	nM	2.810		
		29.10	nM	3.110		
		9.09	nM	0.690		
		2.84	nM	1.900		
		0.80	nM	7.970		
						
59-0159	308.337					
59-0159		100.00	uM	2.790		
		31.25	uM	13.530		
		9.77	uM	4.700		
		3.05	uM	10.910		
		953.67	nM	2.800		
		298.02	nM	9.710		
		93.13	nM	4.830		
		29.10	nM	0.650		
		9.09	nM	5.900		
		2.84	nM	6.610		
		0.80	nM	6.250		
						
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		953.67	nM	6.610		
		298.02	nM	11.380		
		93.13	nM	4.460		
		29.10	nM	3.520		
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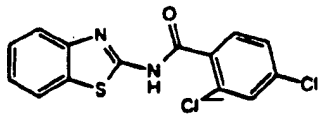						
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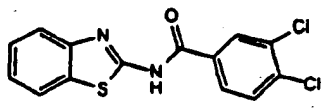
		2.84	nM			
		0.80	nM			
						
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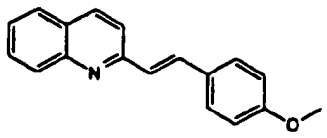
		29.10	nM			
		9.09	nM			
		2.84	nM			
		0.80	nM			
						
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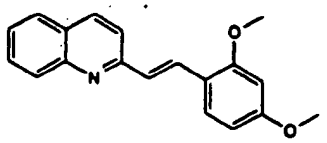
		2.84	nM			
		0.80	nM			
						
59-0199	291.35					
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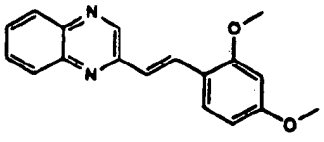						
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		953.67	nM			
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		93.13	nM			
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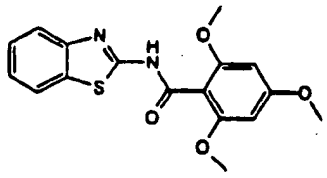						
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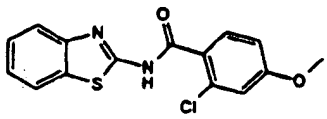						
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		9.09	nM			
		2.84	nM			
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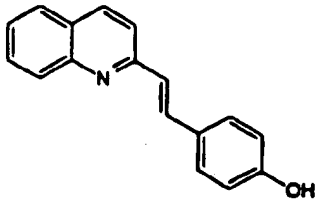						
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		2.84	nM			
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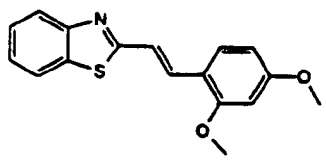						
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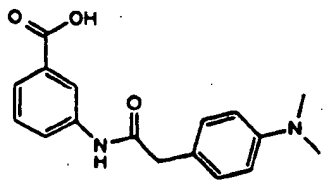						
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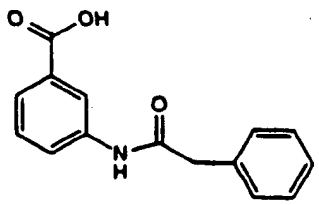						
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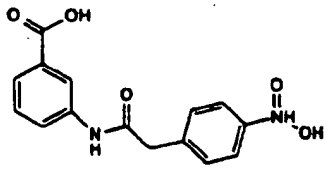						
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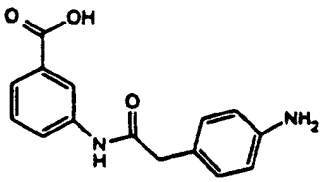						
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		2.84	nM			
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		59XLS	nM			
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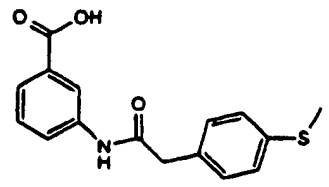						
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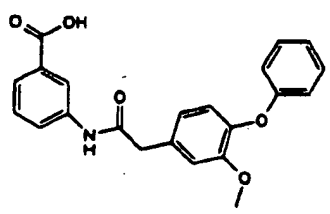						
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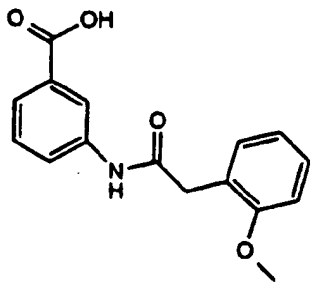						
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		3.05	uM			

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17019

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/54
US CL :514/222.8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/222.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS AND CAS ONLINE: compounds of the claims with bone, osteoporosis, hyperparathyroidism, periodontal, prosthetic, dental

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,280,040 A (LABROO ET AL.) 18 January 1994.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 FEBRUARY 1997	Date of mailing of the international search report 20 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KIMBERLY JORDAN Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*